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The American Phytopathological Society

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THE MODE OF ACTION OF BORDEAUX ON MYCOSPHAERELLA FRAGARIAE

A. G. PLAKIDAS

(Accepted for publication February 1, 1938)

INTRODUCTION

Earlier studies at the Louisiana Agricultural Experiment Station (13, 14) have shown (1) that infection with the strawberry leaf-spot fungus, *Mycosphaerella fragariae* (Tul.) Lindau, takes place primarily, if not wholly, through the dorsal surface of the leaf, and (2) that the disease can be controlled effectively by spraying with Bordeaux mixture, even though, with the system of spraying practiced by the strawberry growers in Louisiana, very little of the spray material actually reaches the dorsal side of the leaves. These two observed facts appear contradictory, since the action of Bordeaux spray is generally assumed to be protective, and yet, in this case control was obtained without protecting the surface of the leaf through which infection occurs.

In the hope of obtaining information that might explain the apparent inconsistency in the results of previous studies, an investigation was undertaken to determine the manner in which Bordeaux spray acts on the conidia of *Mycosphaerella fragariae*. Under Louisiana conditions where the strawberry plants remain active during the winter, the dissemination and perpetuation of the fungus depend almost solely on conidia. The primary object of this investigation was to determine the action of copper compounds, particularly that of Bordeaux mixture, on the conidia. It was felt that a knowledge of the tolerance of these spores for the fungicide would be a contribution to a better understanding of the way in which control is obtained by spraying and might lead to a more efficient method of controlling this important disease. The methods and results of this investigation form the substance of this paper. A brief abstract of the subject has been published (15).

MATERIALS AND METHODS

Source of Spores. The spores used in the various tests were obtained from naturally infected leaves collected in the field. The leaves were washed under running water with a brush and placed in moist chambers. The washing, besides removing the grit and other foreign material and leaving the surface clean, served the additional purpose of removing the old spores so that those spores that were produced in the moist chambers were of approximately the same age when used in the toxicity tests. In this way variability due to age of spores was largely eliminated. In about 24 hours after the leaves were placed in the moist chamber, spores were produced in great profusion. These were picked by means of a closely cropped camel-hair brush and made into the desired suspension. It was found that the most uniform germination was obtained with spores 24 to 36 hours old; spores of this age

were used in most of the tests. The density of the suspension was obtained by making microscopic mounts and examining with an intermediate objective (Zeiss No. 20). Optimum germination was obtained when approximately 10 spores to the field were present.

Water. The spores germinated well (usually over 90 per cent) in tap water and, so, in the tests with Bordeaux mixture, tap water was used for the checks and also for the preparation of the Bordeaux mixture and the lime suspension. However, since the Baton Rouge water is rather strongly alkaline (pH 8.0–8.5), tap water could not be used in the tests involving high dilutions of soluble copper compounds, such as copper sulphate and copper chloride, because precipitation of the copper occurred. Germination in distilled water was erratic. Usually the spores germinated as well in distilled as in tap water; but, occasionally, poor or irregular germination occurred in distilled water. For this reason, rain water (when available) or tap water, rendered slightly acid (pH 6.5) by the addition of hydrochloric acid, was used in the series of tests with high dilutions of soluble copper compounds. The spores germinated well both in rain water and in acidified tap water.

Glassware. The importance of clean glassware in spore germination tests has been well emphasized by McCallan (8) and other workers.

The slides and other glassware were cleaned by boiling in dichromate cleaning mixture then washing successively in several changes of tap water, alkaline water, distilled water, and 95 per cent alcohol. With the slide perfectly clean, the drop of spore suspension placed on it would spread out evenly, forming a thin film that provided uniform aeration conditions; with a slide not thoroughly clean, the suspension would form a round, heavy drop that did not allow uniform aeration.

The slides were placed on glass-rod racks in large (21 cm. diameter) moist chambers, and were examined for germination usually at the end of 24 hours, and in certain cases reexamined at the end of 36 or 48 hours. In the beginning, 8 slides were used for each series. However, since the variation in the percentage of germination between the different slides of a series was found to be very small, the number of slides per series was reduced to 4 and the tests were repeated. The germination counts were made by placing each slide on the mechanical stage of the microscope, counting all the spores that appeared on one microscopic field, then so moving the slide that the same field was not counted twice. Usually 100 spores were counted per slide, so that the average germination was obtained from a count of 400 or 800 spores, depending on whether 4 or 8 slides were used per series.

EXPERIMENTAL

Exploratory tests were first carried out for the purpose of obtaining some leads as to the manner in which Bordeaux acts on *Mycosphaerella fragariae*, for, as already stated, effective control of leaf spot is obtained by spraying with Bordeaux mixture, even though the spray does not cover the under sur-

face of the leaves through which the infection takes place. These preliminary tests were intended primarily to answer the following questions: (1) Does the toxic principle of the spray penetrate the leaf tissue to kill the fungus in it? (2) Are spores produced on the sprayed leaves? (3) If spores are produced on the sprayed leaves, are they viable, *i.e.*, will they germinate if placed in a favorable medium?

EFFECT OF BORDEAUX ON INTERNAL MYCELIUM

The first question was easily answered. Spotted leaves that had been sprayed twice, January 2 and 10, were collected in the field on January 13, brought to the laboratory, and washed thoroughly in running water with a brush to remove the spray residue. Then, pieces of tissue from the younger spots were cut, surface-disinfected in 1–1,000 mercuric chloride for ½ minute, washed with sterile water, and placed on bean-pod agar. The fungus was easily isolated, which made it apparent that the mycelium within the tissue was not killed by the spray.

COMPARISON OF THE NUMBER OF SPORES ON SPRAYED AND NON-SPRAYED LEAVES

The following procedure was followed in making comparative counts of the number of spores on sprayed and nonsprayed leaves: A definite number of spots (usually 5) of as nearly as possible the same size and age were selected from each lot, and their upper surfaces were brushed with a close-cropped camel-hair brush into a watch glass containing 0.5 cc. of water. One drop (1/20 cc.) of the spore suspension was placed on a slide and covered with a 22 mm. cover slip. This amount of spore suspension was just enough to form an even film between the slide and the cover slip. The slide was then placed on the microscope, which was equipped with a mechanical stage, and examined with a Zeiss No. 20 objective, and the spores appearing in a field counted. The slide was then moved to other fields, care being taken not to count the same field twice.

While it is realized that this method is empirical and relatively crude, it is considered satisfactory for purposes of comparison, since the same procedure was followed with both the sprayed and the nonsprayed leaves, and since the many times repeated tests gave consistent results. The following example illustrates the reliability of this method. When 5 spots were brushed to make the spore suspension, the average number of spores per microscopic field (average of 40 fields) was 2.55 and 0.27, respectively, for the nonsprayed and the sprayed leaves; when 10 instead of 5 spots were used from the same lots of leaves, the average number of spores per microscopic field (average of 50 fields) was 4.60 and 0.62, respectively. These figures are roughly double those obtained when 5 spots were used.

From the results of many different tests, it was concluded that relatively few spores are produced on the upper surface of a sprayed leaf. Between 7 and 10 times as many spores were found on the nonsprayed as on the sprayed leaves. It is believed that when moisture is present on the sprayed

leaves, spray hinders normal growth of the sporogenous hyphae, thereby inhibiting sporulation to a great extent and reducing considerably the amount of potential inoculum at its source. In this way, Bordeaux may be considered to act more as an eradicative than as a protective fungicide. Table 1 shows the results obtained from a series of tests that may be considered as representative of many others. The material for this series was

TABLE 1.—*Comparison of the number of spores of Mycosphaerella fragariae on sprayed and nonsprayed strawberry leaves*

Microscopic field	Sprayed				Nonsprayed			
	Test I	Test II (Same suspension as in I, but different slide)	Test III (New suspension)	Test IV (Same suspension as in III, but different slide)	Test I	Test II (Same suspension as in I, but different slide)	Test III (New suspension)	Test IV (Same suspension as in III, but different slide)
1	1	3	1	2	6	10	4	7
2	1	0	1	0	6	3	12	3
3	2	0	0	0	2	6	5	8
4	0	1	0	1	6	4	9	9
5	2	2	4	2	3	8	6	5
6	0	0	0	0	11	2	3	2
7	0	3	2	0	9	5	10	4
8	0	0	1	0	2	9	5	6
9	0	0	0	2	5	6	7	10
10	0	1	0	0	7	7	5	3
	Average for the 4 tests (40 fields) 0.8				Average for the 4 tests (40 fields) 6.00			

collected in the field at Hammond, Louisiana, on January 19, 1935, and consisted of leaves of nonsprayed plants and from plants that had been sprayed twice (January 2 and 10) with a 4–4–50 Bordeaux mixture. The sprayed and nonsprayed plants were of the same variety (Klondike) and were growing on adjoining rows under identical conditions.

VIABILITY OF SPORES PRODUCED ON SPRAYED LEAVES

When sprayed, spotted leaves were placed in a moist chamber with the humidity high enough to stimulate growth, but not so high as to form a film of moisture on the sprayed surfaces, spores were produced in abundance on both the upper and the lower surfaces, those on the upper (sprayed) surface pushing out through the dry spray residue. When such spores were picked at the point of a needle, care being taken not to include any spray particles, and placed in water on slides, they germinated well, showing that they were viable. If, however, the sprayed, spotted leaves in the moist chamber were sprayed with water by means of an atomizer so as to wet the spray residue and form a thin film of moisture on their surfaces, no spores were produced, except on elevated areas where the film of moisture evaporated, leaving the spray residue dry. Where the leaf was level, so that the film of moisture

covered the surface and kept the spray residue wet, no spores were produced either on the upper or under surface. Since no spray material was present on the under leaf surface, it was apparent that some of the copper in solution must have diffused through the dead tissue of the spots and reached the under surface. Furthermore, if the upper surfaces of the sprayed, spotted leaves in the moist chambers were atomized with water after spores had been produced, and allowed to stay for sometime (usually overnight) it was found that the spores on the upper surface were invariably killed, for they never germinated when transferred to water on slides.

The germination of the spores on the under leaf surface was variable. When slides were prepared from separate spots, the percentage of germination varied greatly, from no germination at all, in some cases, to nearly as high as that of the checks in others. This would indicate that the copper in solution does not become equally distributed on the under leaf surface.

That the data obtained from the laboratory tests give a true picture of what happens under field conditions, was brought out by the following experiment. On February 7, 1934, leaves were collected from sprayed (4 times) and nonsprayed plants in the garden in Baton Rouge, brought to the laboratory, and slides for germination tests were prepared immediately. Heavy dew had fallen during the night, and, when the leaves were collected at 8:00 a.m., a thick film of moisture occurred on their upper, and droplets of moisture on their under surfaces. Spores were picked from the under leaf surface only and placed in tap water on slides. Six series of slides (4 slides to each series) were thus prepared from as many separate leaves. Table 2 shows the results obtained.

TABLE 2.—*Comparison of the germination of spores of Mycosphaerella fragariae produced on the under surfaces of Bordeaux-sprayed and nonsprayed leaves under field conditions*

Series	Percentage germination (ave. of 4 slides)	
	Sprayed	Nonsprayed
1	4.2	92.1
2	1.7	88.7
3	61.7	95.4
4	7.2	93.8
5	72.6	96.0
6	9.3	91.8

These results are similar to those obtained with spores produced on leaves in moist chambers in the laboratory and indicate that, even though no spray residue may be visible on the under surfaces of the sprayed leaves, some of the soluble copper must reach and kill at least some of the spores formed on the under surface. That the spores that failed to germinate were already dead, rather than that germination was inhibited by the chance inclusion of some spray residue particles in the spore suspension on the slides, seemed evident from the fact that the germ tubes of the spores that did germinate

developed normally, even in the series where the percentage of germination was very low. For example, in series 2, where the percentage of germination was the lowest (1.7 per cent) two spores had, at the end of 34 hours, long, branched germ tubes, the longest branches of which were 274 μ and 484 μ, respectively.

TESTS WITH BORDEAUX MIXTURE

After the preliminary tests had indicated that *Mycosphaerella* spores are sensitive to copper toxicity, a long series of experiments extending from 1934 to 1937 were carried out in order to determine more accurately the action of Bordeaux mixture on the spores. Various forms of Bordeaux, namely, freshly prepared, aged on glass slides for different periods, freshly sprayed on leaves, and weathered on leaves was used in these experiments.

DIRECT CONTACT WITH THE BORDEAUX PARTICLES

Germination was completely inhibited when the spores came in direct contact with Bordeaux mixture, irrespective of whether it was freshly-prepared, aged on slides, or weathered on leaves. With freshly-prepared mixture, germination was inhibited at as high a dilution as 1 part of a 4–4–50 suspension in 1000 parts of water. The results of a few experiments, which are typical of many performed, are presented.

Experiment I. Tests with Fresh 4–4–50 Bordeaux

A. Check: Spores in tap water. Ave. germination 92.7%; ave. length of germ tubes 157.3 μ.

B. Spores in undiluted and in diluted solutions of 1–100, 1–200, 1–500, and 1–1000 4–4–50 Bordeaux. No germination.

C. Spores in 1–10,000 dilution of 4–4–50 Bordeaux. Ave. germination 84.3%; ave. length of germ tubes 54.7 μ.

D. Spores in 1–20,000 dilution of 4–4–50 Bordeaux. Ave. germination 87.0%; ave. length of germ tubes 86.6 μ.

E. Spores in 1–40,000 dilution. Ave. germination 92.7%; ave. length of germ tubes 159.8 μ.

F. Spores in 1–80,000 dilution. Ave. germination 93.0%; ave. length of germ tubes 161.3 μ.

G. Spores in 1–100,000 dilution. Ave. germination 92.3%; ave. length of germ tubes 153.0 μ.

These results illustrate the extreme sensitivity of the spores of *Mycosphaerella fragariae* to copper toxicity. No germination occurred when the spores were in contact with fresh Bordeaux, even at the high dilution of one part of 4–4–50 Bordeaux suspension in 1000 parts of water; and, although a high percentage of the spores germinated at the 1–10,000 and 1–20,000 dilutions, some degree of toxicity was apparent, even at these extremely high dilutions, as judged by the length of the germ tubes. At the 1–20,000 dilution the average length of the germ tubes was approximately one-half that of the check.

It is admitted, of course, that the concentration of Bordeaux in dilutions is only approximate. It is difficult to dilute coarse suspensions, such as Bor-

deaux mixture, which have variable-size particles, and get the exact concentration of the suspension in the desired dilution. Notwithstanding this difficulty, it is apparent that extremely small amounts of the Bordeaux suspension were present in dilutions of 1–10,000 and 1–20,000; yet this was sufficient to manifest a certain degree of toxicity. No toxic action was apparent in dilutions of 1–40,000 or higher.

Experiment II. Tests with Aged Bordeaux. Measured amounts (1/20 or 0.1 of a cc.) of a 4–4–50 Bordeaux were smeared on glass slides and allowed to dry. The slides were then stored in a slide box for subsequent use. Table 3 shows the plan and the results obtained from a typical test with aged Bordeaux.

TABLE 3.—*Effect of lime and of dried, aged 4–4–50 Bordeaux on the germination of conidia of Mycosphaerella fragariae*

Treatment	Percentage germination (ave of 4 replications)
A. Check—spores in distilled water	95.2 ± 2.12
B. " " " tap "	89.2 ± 5.29
C. Spore suspension on 1/20 cc. 1% lime smear, dried and aged for 5 days. Stirred with a camel-hair brush to mix the spores with the lime particles	94.5 ± 2.07
D. As in "C," but lime smear not stirred	96.0 ± 1.7
E. Spore suspension on 0.1 cc. lime smear; stirred	94.6 ± 1.85
F. " " " " " " " not stirred	95.7 ± 1.48
G. " " " " " Bordeaux smear, dried and aged 5 days; stirred	0
H. As in "G," but not stirred	0
I. Spores on 1/20 cc. Bordeaux smear, aged 5 days; stirred	0
J. " " " " " " " " " not stirred	0
K. " " " " " " aged one year; stirred	0
L. " " " " " " " " " not "	0
M. " " 0.1 " " " " " " stirred	0
N. " " " " " " " " " not "	0

Germination was completely inhibited when a water suspension of spores was placed on dried, aged-Bordeaux smears on glass slides. The results were the same, regardless of the amount of Bordeaux smeared on each slide (1/20 or 0.1 of a cc.) and the period of aging of the smears (5 days or 1 year), and regardless of whether the smear was stirred, so as to bring the spray particles into intimate contact with the spores, or left unstirred.

The lime smears (Table 3, C–F) were included in these tests in order to determine if the lime component of Bordeaux mixture exerted any toxic action against the spores. Several workers have found lime in certain concentrations to be toxic to fungi and bacteria. Lutman (7) noted lime toxicity to spores of *Phytophthora omnivora* and *Sclerotinia cinerea*. McCallan (9) found that C.P. calcium hydroxide in concentrations higher than 0.1 gm. per liter and C.P. calcium sulphate in concentrations higher than 0.7 gm. per liter were toxic to spores of *Sclerotinia americana*; and Keitt, Pinckard, and Riker (6) have shown that a 5-minute exposure of *Erwinia amylovora* to

M/2.16 calcium hydroxide was lethal. As shown in table 3, lime was not toxic to *Mycosphaerella* spores. The percentages of germination in the lime series were as high as those of the water checks, and the germ tubes were long and normal.

Both freshly prepared 4–4–50 Bordeaux and freshly slaked 1 per cent lime were found to have the same reaction, pH 12.1 (determinations made by means of the Coleman glass electrode). After aging (sprayed on glass, allowed to dry, and age for 5 days in the laboratory, then suspended in a volume of water equal to the original) the pH of both 4–4–50 Bordeaux and 1 per cent lime dropped to 9.5. This relatively high alkalinity evidently had no adverse influence on the germination of the spores.

Experiment III. Tests with Weathered Bordeaux. Goldsworthy and Green (5) have noted that the toxicity of Bordeaux mixture to conidia of *Sclerotinia fructicola* diminishes with the exposure of the spray residue to field weathering and rainfall. In order to determine the effect of weathered Bordeaux on the spores of *Mycosphaerella fragariae* sprayed strawberry leaves were collected on December 18, 1936, dried, and stored in paper bags in the laboratory. The last spraying of the plants from which the leaves were collected was done on October 29, 1936, so the spray had been exposed to weathering in the field for 50 days. One part of the field was sprayed with 4–4–50 Bordeaux alone and the other with the same mixture to which an adhesive clay ("Wyo-Jel") was added at the rate of 10 pounds per 100 gallons of spray. On the date of collecting, the spray residue was barely visible on the leaves sprayed with Bordeaux alone, while considerable residue remained on those sprayed with the Bordeaux–"Wyo-Jel" mixture.

On February 5, 1937, these tests were made using the dried, stored leaves:

(1) Spray residue was brushed off the dried leaves and suspensions were prepared using 0.1 g. of the residues in 2 cc. of distilled water. Spores were placed on slides in these suspensions.

(2) Portions of the dried leaves were crumpled into Syracuse dishes in 6 cc. of water, let steep for 36 hours, then filtered through filter paper. Spore suspensions were made in the filtrates and placed on slides in moist chambers.

The results of the tests with weathered Bordeaux are shown in table 4. Germination was completely inhibited by Bordeaux spray residue that had weathered on leaves outdoors for 50 days and aged in the laboratory for an additional period of 49 days. The residue had been exposed to 4.69 inches of rain—it rained 4 times (total 2.07 inches) in November and 5 times (total 2.62 inches) in December. That the spores germinated relatively well in the filtrate of the leaves sprayed with the mixture containing the "Wyo-Jel" clay, while they did not germinate at all in the filtrate of the leaves sprayed with Bordeaux alone was probably due to the fact that the clay acted as a filter, adsorbing and taking out of solution the small amount of soluble copper present. The filtrate of the leaves sprayed with Bordeaux alone was dark brown, while that of the leaves sprayed with the Bordeaux–"Wyo-Jel" mixture was nearly colorless, indicating that even the color of the solution was adsorbed by the "Wyo-Jel" clay.

TABLE 4.—*Effect of weathered Bordeaux mixture residue on the germination of conidia of Mycosphaerella fragariae*

Treatment	Percentage germination (ave. of 4 replications)
A. Check—spores in tap water	93.2 ± 1.8
B. Check—spores in distilled water	91.5 ± 2.7
C. Spores in weathered Bordeaux residue	0
D. Spores in weathered Bordeaux plus "Wyo-Jel" residue	0
E. Spores in filtrate of nonsprayed leaves	94.1 ± 2.06
F. Spores in filtrate of leaves sprayed with Bordeaux alone	0
G. Spores in filtrate of leaves sprayed with Bordeaux plus "Wyo-Jel"	83.3 ± 6.3[a]

[a] The percentage of germinated spores differed considerably between the 4 slides of this series, as seen by the large standard deviation, and the germ tubes were shorter than those of the checks; otherwise, the germination was normal.

Experiment IV. Spore Germination on Leaves. All the germination tests reported above were made on glass slides, *i.e.*, under unnatural conditions. In order to simulate field conditions as nearly as possible the following experiment was carried out. Strawberry leaves were collected in the field on March 4, 1937, and divided into 3 lots, *viz.*:

Lot 1. Nonsprayed leaves.

Lot 2. Leaves sprayed with 4–4–50 Bordeaux on February 22, *i.e.*, after 10 days of weathering.

Lot 3. Leaves sprayed last on March 4, collected about 2 hours after spraying, just as soon as the spray had dried thoroughly.

The leaves were placed in Petri dishes in moist chambers, care being taken not to disturb the spray residue film, and a few drops of a suspension of spores of *Mycosphaerella fragariae* in tap, rain, or distilled water were placed on their surfaces. After 24 hours, representative samples of the spore suspensions were transferred by means of a pipette from the leaves onto glass slides and examined for germination (Table 5). No germination occurred on the

TABLE 5.—*Germination of conidia of Mycosphaerella fragariae on the surface of Bordeaux-sprayed and nonsprayed strawberry leaves*

Treatment	Percentage germination (ave. of 6 replications)
A. Check—spores in distilled water on nonsprayed leaves	72.6
B. Check—spores in tap water on nonsprayed leaves	75.2
C. Check—spores in rain water on nonsprayed leaves	88.0
D. Spores in distilled water on leaves sprayed Feb. 22 (10 days' weathering)	0
E. Spores in tap water on leaves sprayed Feb. 22 (10 days' weathering)	0
F. Spores in rain water on leaves sprayed Feb. 22 (10 days' weathering)	0
G. Spores in distilled water on leaves sprayed March 4 (no weathering)	0
H. Spores in tap water on leaves sprayed March 4 (no weathering)	0
I. Spores in rain water on leaves sprayed March 4 (no weathering)	0

sprayed leaves. The spores appeared dead, with their contents plasmolyzed and coagulated. The percentage of germination in the checks (nonsprayed leaves) was lower than that usually occurring on glass slides, but this is believed to be due to the fact that spores with long germ tubes would tend to adhere to the leaf surface, and, in transferring the spore suspension from the leaf to the glass slide, a larger proportion of nongerminated spores would be picked by the pipette.

In order to determine if the dead-appearing nongerminated spores (Series D–I, Table 5) were actually killed or if germination was merely inhibited, the suspensions from several leaves from series D, E, and F and from series G–I, respectively, were combined on filter paper in a funnel and the spores were washed for ½ hour with running water. The spores were then placed on slides and examined 24 hours later to see if they would germinate. Checks were provided (1) by placing spores in the last washings of the Bordeaux-exposed spores and (2) by washing fresh spores in water on filter paper for the same length of time as the Bordeaux-exposed spores. Good germination was obtained in both checks. The Bordeaux-exposed, washed spores did not germinate. This indicates that they were actually killed rather than that germination was inhibited by a 24-hour exposure to Bordeaux residue on leaves.

ACTION OF BORDEAUX AT A DISTANCE

Under this heading are discussed, for the sake of convenience, miscellaneous experiments in which the spores were not brought in direct contact with the spray particles, but were either separated from them by a water bridge or by 1 or 2 thicknesses of paper, or were exposed to the supernatant, clear liquid of fresh or dried Bordeaux.

TOXIC ACTION OF BORDEAUX THROUGH WATER BRIDGE

That Bordeaux can exert its toxic action at a distance from its solid particles through a film of water has been shown by Aderhold (1), Lutman (7), McCallan (9), and others. Barker and Gimingham (3), on the other hand, have maintained that actual contact, or at least very close association, is necessary for toxic action. McCallan's (9) method was used to determine if Bordeaux was toxic to conidia of *Mycosphaerella fragariae* at a distance.

One drop (approx. 0.05 cc.) of a 4–4–50 Bordeaux mixture was placed on each of several slides and allowed to dry for a week. A spore suspension in distilled water was prepared and a drop of it was placed on the same slides at distances of 1, 2, 5, and 10 mm. from the Bordeaux spot. After waiting for about 10 minutes to allow the spores to settle, a drop of tap water was placed on the Bordeaux spot, and a narrow bridge of water (approx. 2 mm. in width) was made by means of a fine pipette connecting the drop of spore suspension with the drop of water on the Bordeaux spot. In one series, 2 drops of spore suspension were placed on the slides, the first drop 3 mm. from the Bordeaux spot and the second 3 mm. from the first. The first spore-

suspension drop was connected to the Bordeaux spot on one side and to the second spore drop on the other by means of a narrow bridge or film of water, as shown in figure 1. The drops were approximately 10 mm. in diameter.

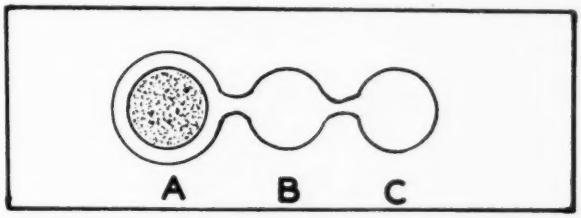

FIG. 1. Diagram showing the arrangement of the Bordeaux smear and the two drops of spore suspension on the slide. A. Bordeaux smear and film of water over it. B. First spore drop located 3 mm. from the Bordeaux smear and from the second spore drop (C) and connected with them by narrow water-film bridges. The diameter of the spore drops was approximately 10 mm., so the distance between the Bordeaux smear and the second spore drop was approximately 16 mm.

Therefore, the second spore drop was approximately 16 mm. from the Bordeaux spot. In making the water bridges, care was taken to move the pipette from the spore suspension drop toward the Bordeaux spot so as to prevent any loose Bordeaux particles from floating toward the spore drops. The slides were placed in moist chambers and examined 24 hours later.

In connection with this experiment, spore suspensions were made in the supernatant clear liquid of freshly prepared 4–4–50 Bordeaux, and also in the supernatant liquid of dried 4–4–50 Bordeaux. The latter was obtained by placing small amounts of a 4–4–50 Bordeaux mixture in Syracuse watch glasses, so rotated that the sides and bottoms became well coated, and allowed to dry and age for a week. Distilled water was placed in the Bordeaux-

TABLE 6.—*Germination of conidia of Mycosphaerella fragariae in water connected by means of a water-film bridge with a spot of dried Bordeaux and in the supernatant liquids of fresh and dried 4–4–50 Bordeaux*

Treatment	Results
A. Check—spores in tap water	Ave. germination 94.6 ± 1.8
B. Check—spores in distilled water	" " 87.7 ± 2.3
C. Spore drop 1 mm. from Bordeaux	No germination; spores unchanged.
D. Spore drop 2 mm. from Bordeaux	No germination; spores unchanged.
E. Spore drop 5 mm. from Bordeaux	No normal germination; spores swollen, some with "buds"[a] (Fig. 2, D)
F. Spore drop 10 mm. from Bordeaux	No normal germination; spores swollen, some with short germ tubes (Fig. 2, E)
G. Two separate spore drops connected to the Bordeaux spot in series as shown in figure 1	No germination in first drop. In the second drop germination was approx. same as in series "F."
H. Spores in supernatant liquid of fresh Bordeaux	No germination.
I. Spores in supernatant liquid of dried Bordeaux	No normal germination; spores swollen, some with short, curled, twisted germ tubes (Fig. 2, C)

[a] The term "bud" is used in the sense used by Goldsworthy and Green (5) to indicate the primordial germ tube or the beginning of germination.

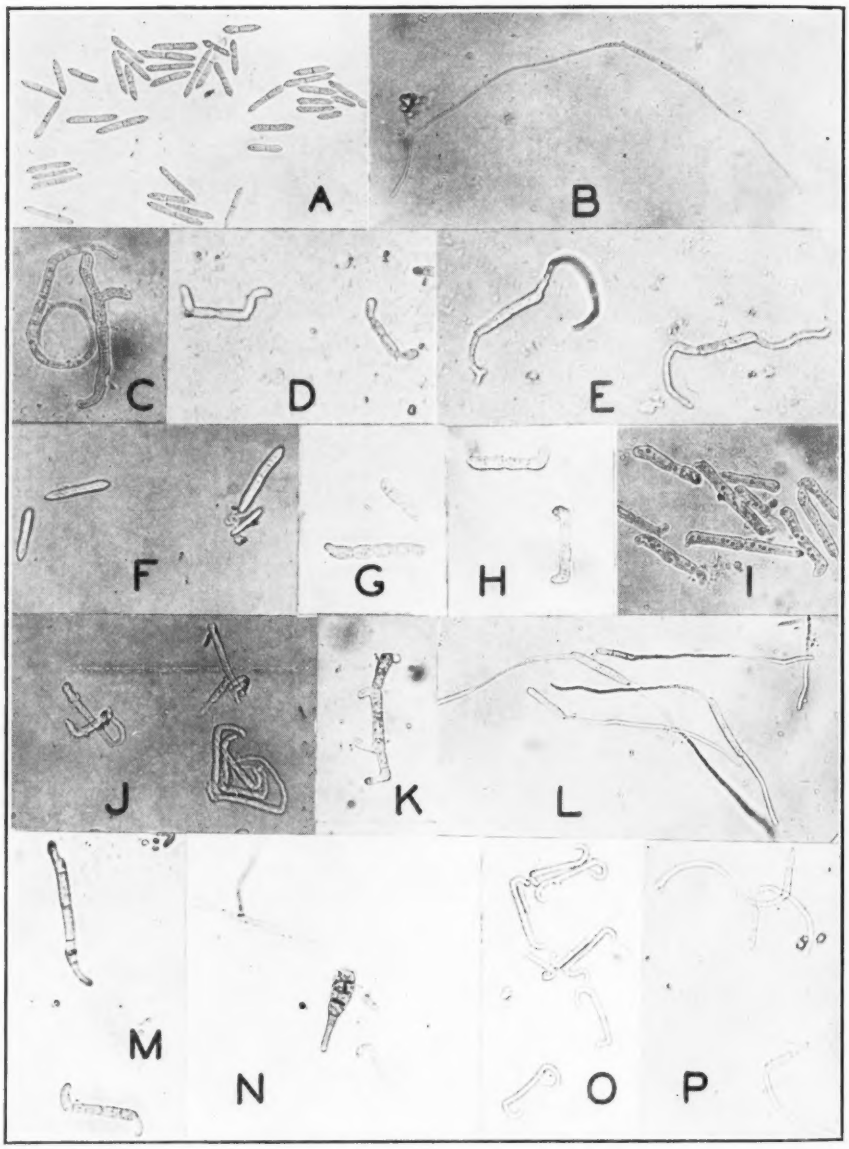

FIG. 2. Copper toxicity to conidia of *Mycosphaerella fragariae*. A. Normal ungerminated 24-hour-old conidia. ×300. B. Normal germination in tap water in 24 hours, showing long and relatively straight germ tubes. ×330. C. Germination in the supernatant liquid of dried Bordeaux in 24 hours. Note the extreme swelling of the conidia and the peculiar curling of the germ tubes. ×330. D. Germination at a distance of 5 mm. from the Bordeaux smear in 24 hours. The growth of the germ tubes did not go beyond the "bud" stage. ×330. E. Germination at a distance of 10 mm. from the Bordeaux smear in 24 hours. Short germ tubes were formed, but no further growth occurred. ×300. F. Conidia in 4 p.p.m. of $CuSO_4$. No germination and no swelling in 24 hours. ×330. G. Swelling but no germination of conidia in 3.33 p.p.m. of $CuSO_4$ in 24 hours. ×330. H. Swelling and "budding" but no normal germination in 2 p.p.m of $CuSO_4$ in 24 hours. ×330. I. Conidia in 1 p.p.m of $CuSO_4$. Swelling and "budding" but no normal germination in 24 hours. ×380. J. Abnormal germination of conidia in 0.5 p.p.m. of $CuSO_4$ in 24 hours. Note the curling and twisting of

coated dishes and allowed to stand for 48 hours, then some of the clear supernatant liquid was drawn off by means of a pipette and used to make spore suspensions. The results of these tests are summarized in table 6. No germination and no swelling of the spores occurred in the supernatant liquid of fresh Bordeaux, and when the spore-suspension drop was 1 or 2 mm. from the Bordeaux spot. At 5-mm. distance, the spores swelled considerably and some began to germinate, but growth stopped at the "bud" (primordial germ tube) stage (Fig. 2, D). At 10-mm. distance, approximately 40 per cent of the spores germinated, but the growth of the germ tubes soon stopped; the germ tubes were no longer at the end of 30 hours than at the end of 24 hours. Germination was not normal in the supernatant liquid of dried Bordeaux (Table 6, I). The spores swelled greatly, and those that germinated had thickened, curled, twisted germ tubes (Fig. 2, C). This peculiar type of germination has been observed in all cases in which the concentration of copper in the solution was on the border line—not enough to inhibit germination but enough to prevent normal growth of germ tubes. A spore germinating normally in water swells somewhat but not greatly, a germ tube being pushed out from one end, and a little later a second one from the other end. The 2 tubes grow out relatively straight (Fig. 2, B) without curling or twisting, and usually no branching occurs during the first 24 to 30 hours. In copper solutions several characteristic abnormalities have been noted. In relatively high concentrations (1–200,000 copper sulphate, for example) the spores are killed and no germination occurs. In higher dilutions (1–200,000 to 1–1,000,000 copper sulphate, for example) the spores swell and enlarge greatly, and "buds" of germ tubes appear, but no further growth takes place (Fig. 2, G–I). Often germ-tube "buds" develop not only from the ends of the spores but also from their sides (Fig. 2, C, K, N, O). Germination from the sides of the spore has never been observed in the checks. In still higher dilutions (those approaching the upper limits of toxicity, 1–2,000,000 copper sulphate, for example) some germination occurs, but growth of the germ tubes soon stops, and the germ tubes curl in a very characteristic manner (Fig. 2, J, O). The reason for this peculiar curling of the germ tubes has not been ascertained.

On the slides containing 2 drops of spore suspension connected in series to the Bordeaux spot (Fig. 1), no germination occurred in the first drop (3 mm. from the Bordeaux spot); in the second drop (16 mm. from the Bordeaux spot) the spores were swollen and about 50 per cent had germinated with short germ tubes. It is evident that where the spores germinated at a distance (5, 10, and 16 mm., Table 6, E–G) germination occurred before suffi-

the short germ tubes. ×330. K. Germination in 0.67 p.p.m. of $CuCl_2$ in 24 hours. Note the formation of germ-tube buds both from the ends and the sides of the conidium. ×330. L. Normal germination of conidia in 0.25 p.p.m. of $CuSO_4$. ×300. M. Germination in 0.5 p.p.m. of $Cu(CH_3COO)_2 \cdot H_2O$ in 24 hours. ×380. N. Germination in 0.5 p.p.m in $Cu(CH_3COO)_2 \cdot H_2O$ in 24 hours. The *Alternaria* sp. spore germinated well in this dilution, while the *Mycosphaerella fragariae* spore produced only short "buds." ×330. O and P. Germination in 0.33 p.p.m. of $Cu(CH_3COO)_2 \cdot H_2O$ after 24 and 30 hours, respectively. ×330.

cient copper ions had diffused through the water bridge to inhibit further growth of the germ tubes; for, the greater the distance between the spore suspension and the Bordeaux spot the longer were the germ tubes.

At the end of 24 hours, half of the slides in each of series F, G, and I, table 6, were treated with a drop of 1–1,000 mercuric chloride to stop further growth of the germ tubes and the other half were left untreated for comparison, and all were reexamined at the end of another 24-hour period. No elongation of the germ tubes occurred in series F and G (10 mm. and 16 mm., respectively, from the Bordeaux spot). Some further growth of the germ tubes occurred in series I (supernatant liquid of dry Bordeaux), though it was not normal, the germ tubes being much shorter than those of the checks and considerably curled and twisted. No attempt was made to determine if the higher toxicity exhibited by the distilled water in contact with the dry Bordeaux (Series F and G, Table 6) relative to the supernatant liquid of dry Bordeaux (Series I, Table 6) was due to any solubilizing action of the conidia on the Bordeaux, as shown by McCallan (9) and McCallan and Wilcoxon (10) for the spores of other fungi, or to possible cumulative action of the copper. McCallan (9) attributed the toxic action of filtrate from dried Bordeaux to conidia of *Sclerotinia americana* to soluble calcium salts or to the alkalinity of the filtrate. In the studies here reported the toxicity of the dry Bordeaux filtrate cannot be attributed to soluble calcium or to alkalinity, because it was found that *Mycosphaerella* conidia germinated well in a suspension of lime (Table 3). Tests with chromotropic acid (2) showed the presence of copper in the supernatant liquid of dry Bordeaux, although the amount was not definitely determined. McCallan and Wilcoxon (10) found 0.2 to 0.3 p.p.m. of copper in distilled water that had remained in contact with dry Bordeaux in glass chambers overnight. In these tests, the amount of soluble copper in the supernatant liquid of dry Bordeaux was probably less than that found by McCallan and Wilcoxon, because the type of germination obtained in the filtrate was approximately the same as that in 0.5 p.p.m. of copper sulphate (0.127 p.p.m. metallic copper).

ACTION OF BORDEAUX WHEN SEPARATED FROM THE SPORES BY FILTER PAPER

In the preceding discussion, it has been brought out that germination was completely inhibited in those tests in which conidia of *Mycosphaerella fragariae* were brought in direct contact with suspensions of Bordeaux mixture, regardless of whether the mixture was freshly prepared, dried, and aged on glass slides, or sprayed and weathered on leaves. However, results of this type do not indicate whether the spores that do not germinate are actually killed or whether germination is merely inhibited.

Clark (4) has called attention to the difference between *point of inhibition of germination* and *death point*, and has emphasized the difficulty of separating the fungus spores from the fungicide, which is necessary if the death point is to be determined. He concluded that inhibition of germination is the important point to consider, as his studies indicated that ". . . if the

germination of the spores be inhibited by a concentration of copper salt, it was merely a question of time when they would be killed." Goldsworthy and Green (5) also distinguished between inhibitory and lethal effects of spray residues.

Some tests were conducted to determine if the spores of *Mycosphaerella fragariae* that failed to germinate when exposed to Bordeaux were actually killed or whether germination was merely inhibited. A small amount of spore suspension (0.5–1.0 cc.) was put in a watch glass, a filter paper of slightly larger diameter than the watch glass was placed over it, then a Bordeaux suspension of the desired dilution was placed on the filter paper. The process was reversed in some of the experiments, *i.e.*, the Bordeaux suspension was placed in the watch glass and the spores on the filter paper. After the desired exposure, the spores were transferred to filter paper in a small funnel, washed thoroughly with tap water, placed on slides in a moist chamber, and examined for germination at the end of 24 hours. Naturally, many of the spores were lost during the process of washing and transferring to the slides, but enough remained for germination counts.

In the beginning, one thickness of filter paper was used, but fearing that some Bordeaux particles might adhere to the paper and get on the slides while the washed spores were being transferred from the filter paper to the slides, 2 layers of filter paper were used in most of the later tests. Where high dilutions of Bordeaux were used (Experiments 8 and 9, Table 7), the method was further modified. The spore suspension was placed on filter paper in a funnel, so that the water would drain and leave the spores within the cone of the filter. The paper was then carefully folded, so as to prevent the escape of the spores, and placed on the bottom of a Syracuse dish. A second piece of filter paper, larger than the inside diameter of the dish, was cupped and placed over the folded paper in the dish, and 10 cc. of the desired dilution of Bordeaux were poured in the dish on the cupped paper. The spores were thus separated from the Bordeaux by 2 thicknesses of filter paper.

Three kinds of checks were provided: (1) Spores in water were washed in the same manner and for the same length of time as were the Bordeaux-exposed spores; (2) Fresh spores in tap water on slides, not washed; (3) Fresh spores in water from the last washing of the Bordeaux-exposed spores. For lack of space, only a few of the results with the checks are included in the summary of the experiments (Table 7), but germination in all cases was high (over 90 per cent).

The method has the serious disadvantage that it does not give a true picture of what happens when there is no partition to separate the spores from the Bordeaux particles. When spores were placed in a suspension of Bordeaux mixture, they have been observed to adhere to the Bordeaux particles, so that there was a very close association between them and the fungicide, an association not attained when a paper partition separates the spores from the Bordeaux particles. Nevertheless, the method proved useful in demonstrating the high sensitiveness of *Mycosphaerella fragariae* conidia

TABLE 7.—*Effect of concentration of Bordeaux and time of exposure on the germination of conidia of Mycosphaerella fragariae when the spores were separated from the Bordeaux particles by means of one or two thicknesses of filter paper. After the desired exposure, the spores were washed on filter paper, transferred to tap water on slides in moist chambers, and examined 24 hours later for germination*

No. and date of experiment	Material[a] and arrangement	Dilution	Period of exposure	Results (percentage germination; average of 4 replications)
1	A	———	17 hrs.	0.0
Jan. 13, 1934	Check[b]	———	——	94.2
	Check[c]	———	——	91.6
2	B	1–21	½ hr.	0.0
Feb. 12, 1934		1–21	1 hr.	0.0
		1–21	2 hrs.	0.0
		1–21	3 hrs.	0.0
	Check[b]	———	——	91.8
	Check[c]	———	——	90.8
3	C	1–21	1½ hrs.	0.0
Feb. 14, 1934		1–21	3 hrs.	0.0
4	D	1–21	½ hr.	0.0
Feb. 14, 1934		1–21	1 hr.	0.0
		1–21	3 hrs.	0.0
5	B	1–20	5 min.	55.5 (germ tubes short)
Feb. 10, 1935		1–30	5 min.	6.1 (" " ")
		1–50	5 min.	11.4 (" " ")
		1–100	5 min.	38.0 (" " ")
		Undiluted	30 min.	0.0
	Check[b]	———	——	93.2 (" " long)
6	E	1–24	15 min.	0.0
Feb. 15, 1935		1–24	30 min.	0.0
		1–44	30 min.	35.0 (germ tubes short, gnarled)
		1–10	60 min.	0.0
7	C	Undiluted	30 min.	0.0
March 4, 1935		1–25	30 min.	8.2[d] (germ tubes as long as those of check)
		1–50	30 min.	0.0 (spores swollen)
		1–100	30 min.	0.0 (" ")
		1–10	5 min.	86.5 (germ tubes shorter than those of check)
		1–20	5 min.	91.5 (germ tubes shorter than those of check)
		1–50	5 min.	95.5 (germ tubes as long as those of check)
		1–20	10 min.	52.0 (only "buds" of germ tubes)
		1–40	10 min.	81.0 (germ tubes long)
		1–80	10 min.	89.0 (" " ")
		1–100	10 min.	85.0 (" " ")
8	C	1–5,000	18 hrs.	63.0 (ave. length of tubes 44.7μ)
March 7, 1935		1–2,000	18 hrs.	51.0 (" " " " 84.0μ)
		1–1,500	18 hrs.	59.5 (" " " " 61.2μ)
		1–1,000	18 hrs.	43.0 (" " " " 62.2μ)
		1–500	18 hrs.	29.0 (" " " " 22.4μ)
	Check[b]	———	——	91.5 (" " " " 97.8μ)

TABLE 7.—(*Continued*)

No. and date of experiment	Material[a] and arrangement	Dilution	Period of exposure	Results (percentage germination; average of 4 replications)
9 March 21, 1935	C	1–500	18 hrs.	54.0 (ave. length of tubes 30.6μ)
		1–400	18 hrs.	61.5 (" " " " 35.7μ)
		1–300	18 hrs.	43.0 (" " " " 43.3μ)
		1–250	18 hrs.	46.0 (" " " " 28.3μ)
		1–200	18 hrs.	38.0 (" " " " 31.5μ)
		1–150	18 hrs.	41.0 (" " " " 22.8μ)
		1–100	18 hrs.	0.0 (spores swollen)
		1–50	18 hrs.	0.0 (no swelling of spores)
		1–25	18 hrs.	0.0 (" " " ")
	Check[b]	———	——	90.5 (ave. length of germ tubes 131.9μ)
	Check[c]	———	——	93.6 (ave. length of germ tubes 142.0μ)

[a] A = Weathered (10 days) Bordeaux residue from leaves in watch glass, separated from spore suspension by filter paper. B = Fresh 4–4–50 Bordeaux separated from spores in watch glass by filter paper. C = Fresh 4–4–50 Bordeaux separated from spores in watch glass by two layers of filter paper. D = 4–4–50 Bordeaux dried and aged on glass slides for 1 week, separated from spores in watch glass by 2 layers of filter paper. E = 4–4–50 Bordeaux, dried and aged on glass slides for 1 year, separated from spores in watch glass by filter paper.

[b] Spores in tap water, washed.

[c] Fresh spores in water from last washing of Bordeaux-exposed spores.

[d] A few of the spores apparently escaped exposure, because there was no gradation in the germination. Of 400 spores examined, 33 were found with germ tubes as long as those of the check; the rest were nongerminated.

to copper toxicity. The results of several experiments with various dilutions of Bordeaux and different periods of exposure are presented in table 7. The outstanding fact brought out by these experiments is that Bordeaux is lethal to the nongerminated conidia, even at relatively short exposures. The spores were killed (or sufficiently injured so that they did not germinate when removed from the fungicide and washed) in every case when exposed to the nondiluted, and to dilute solution of 1–21 Bordeaux for 30 minutes or longer, regardless of whether the Bordeaux was fresh or dried on slides (Experiments 1–7, Table 7). In experiment No. 6 (Table 7), killing of spores occurred at 15 and 30 minutes' exposure to 1–24 dilution of Bordeaux that had been dried and aged on glass slides for one year, and a 30-minute exposure to 1–44 dilution resulted in reduced (35 per cent) and abnormal (short, gnarled germ tubes) germination. In experiment No. 7 (Table 7) no germination occurred after a 30-minute exposure to as high a dilution as 1–100 of freshly prepared 4–4–50 Bordeaux, although the spores were not killed, judging by their swelling after washing. Exposures of 5 to 10 minutes were not lethal, although a certain degree of injury to spores was apparent, even at these short exposures. In one series of tests (Experiment 5, Table 7) injury (as shown by the low percentage of germination and the short germ tubes) resulted by exposing the spores for 5 minutes to dilutions of 1–20, 1–30, 1–50, and 1–100. In another series (Experiment 7, Table 7) the 5-minute exposure had no appreciable effect on the viability of the spores (except for

the shorter germ tubes in the 1–10 and 1–20 dilutions), while a 10-minute exposure to a dilution of 1–20 had a decided effect both on the percentage and type of germination; only 52 per cent of the spores germinated and the germ tubes did not grow beyond the "bud" stage. The tests with the high dilutions of Bordeaux (Experiments 8 and 9, Table 7) were included in order to determine if long exposures to high dilutions are lethal, since it was found earlier that no germination occurred when the spores were in direct contact with a 4–4–50 Bordeaux suspension diluted 1 part in 1,000 parts of water. As seen in table 7, injury resulted from an 18-hour exposure in all dilutions tested (1–25 to 1–5,000) as shown by the reduction in the percentage germination and the length of the germ tubes.

TOXICITY OF SOLUBLE COPPER SALTS

In order to obtain more exact information on the limits of copper toxicity toward *Mycosphaerella fragariae* conidia than was possible when Bordeaux suspensions were used, dilute solutions of copper sulphate ($CuSO_4 \cdot 5H_2O$), copper chloride (anhydrous $CuCl_2$), and copper acetate ($Cu(CH_3CO_2)_2 \cdot H_2O$) were prepared and their action on germination was tested. Two types of tests were made: (1) A spore suspension (density of approximately 10 spores per intermediate-power microscopic field) was made in the desired dilution, and a few drops of it placed on slides in a moist chamber and examined for germination 24 hours later. (2) A spore suspension of approximately the same density as above was made in 2 cc. of the desired dilution in a vial and allowed to stand for 24 hours. The spores were then transferred to a folded filter paper in a small funnel, washed with several changes of water, placed in a few drops of water on slides in moist chambers, and examined 24 hours later for germination. The majority of the tests were made with copper sulphate and copper chloride; only a limited number of tests were made with copper acetate. The results of these tests may be summarized as follows:

I. On Slides in the Respective Dilutions, Spores Not Washed. 1. No germination, no swelling of spores in dilutions of 1–250,000 or lower of copper sulphate, 1–500,000 or lower of copper chloride, and 1–400,000 or lower of copper acetate (Fig. 2, F).

2. In dilutions of 1–300,000 to 1–500,000 of copper sulphate, 1–500,000 to 1–1,000,000 copper chloride, and 1–500,000 to 1–800,000 copper acetate, the conidia swelled considerably (Fig. 2, G) but did not germinate.

3. In dilutions of 1–500,000 to 1–1,000,000 of copper sulphate, 1–1,000,000 to 1–3,000,000 of copper chloride, and 1–1,000,000 to 1–2,000,000 of copper acetate, the majority of the conidia produced very short "buds" of germ tubes (Fig. 2, H, I, K, M). No further growth was noted when the slides were reexamined 24 hours later.

4. In dilutions of 1–2,000,000 copper sulphate, 57 per cent of the conidia germinated with short, twisted, curled germ tubes. Some elongation of the germ tubes was noted (some approaching normal growth) when the slides were reexamined 24 hours later. The same type of germination occurred in

1–3,000,000 copper acetate (Fig. 2, O, P), and 1–4,000,000 copper chloride. The toxic action was still evident at as high a dilution as 1–3,000,000 of copper sulphate (0.085 p.p.m. metallic copper). In this dilution, and also in 1–6,000,000 copper chloride (0.078 p.p.m. metallic copper) and 1–4,000,000 copper acetate (0.0796 p.p.m. metallic copper) the germination was high, 76.0 per cent, 77.2 per cent, and 85.0 per cent, respectively, but still not normal, the germ tubes being much shorter than those of the checks and most of them gnarled, twisted, and curled.

5. Normal germination (Fig. 2, L) was obtained in dilutions of 1–4,000,000 copper sulphate (0.0636 p.p.m. metallic copper). Dilutions of copper chloride higher than 1–6,000,000 and of copper acetate higher than 1–4,000,000 were not used; thus, the limits at which normal germination occurs in these compounds were not determined.

II. Spores Washed After a 24-hour Exposure to the Copper Solution. Only copper sulphate in dilutions of 1–100,000 to 1–2,000,000 was used in this experiment and only 2 separate tests were made. No germination and no swelling of spores occurred in dilutions of 1–500,000 or lower. In one test in which acidified tap water was used in making the dilutions, no swelling of spores occurred in 1–600,000 dilution, while in the 1–1,000,000 dilution most of the spores were swollen but not germinated. In the second test, in which the dilutions were made with rain water, 14.5 per cent of the spores were merely swollen and 10.5 per cent were swollen and had short "buds" of germ tubes in the 1–600,000 dilution. In the 1–1,000,000 dilution 11.5 per cent of the spores were swollen and had short "buds," and 20.0 per cent had short gnarled, curled germ tubes. In the 1–2,000,000 dilution, the type of germination was approximately the same in both tests. There was an average germination of 82.0 per cent, but the germ tubes varied in length from mere "buds" to short, gnarled, curled germ tubes. Many of the spores had multiple germ tubes (germination from the ends and from the sides); the germination at this high dilution of copper sulphate was decidedly abnormal. It would appear from these results that washing did not remove the toxic action of copper. Goldsworthy and Green (5) have shown that, once the copper has been absorbed by the cell, no amount of washing will remove it.

As seen from the results of the tests with soluble copper salts, the limits of toxicity of the different dilutions are not sharply defined. For example, there was very little difference in the appearance of the spores in dilutions of copper sulphate of 1–500,000, 1–600,000, 1–800,000, and 1–1,000,000. In all these dilutions, the spores swelled greatly and produced short "buds" of germ tubes but made no further growth. It is a moot question whether to consider as the critical limit of toxicity (1) the highest dilution in which the germination does not proceed beyond the "bud" stage of germ-tube formation, (2) the one in which abnormal germination (short, gnarled, curled germ tubes) occurs, or (3) one just below the highest dilution in which the germination is normal. If the third is taken as the critical point, then the limit of copper toxicity toward conidia of *Mycosphaerella fragariae* lies somewhere

between dilutions of copper sulphate of 1–3,000,000 (0.085 p.p.m. metallic copper) and 1–4,000,000 (0.0636 p.p.m. metallic copper). Infection most likely will not take place if the concentration of available copper on the leaves is such as to inhibit germination beyond the "bud" stage, and it is doubtful that it will occur under conditions in which germination is abnormal.

COMPARISON BETWEEN CONIDIA OF MYCOSPHAERELLA FRAGARIAE AND THOSE OF OTHER FUNGI IN THEIR SENSITIVENESS TO COPPER TOXICITY

The extreme sensitivity of *Mycosphaerella* conidia to copper toxicity may be illustrated by comparing it with that of spores of some other fungi. In one of the experiments with copper chloride, conidia of *Botrytis* sp. (probably *B. cinerea*, taken from a decaying stem of lupine) were included in the 1–300,000 and 1–500,000 series of dilution. Germination of 52.0 per cent and 56.0 per cent, respectively, was obtained in these dilutions (93.5 per cent in tap water). The germ tubes were also long and apparently normal. The *Mycosphaerella fragariae* conidia, on the other hand, not only did not germinate in these dilutions, but, from all appearances (failure to swell and coagulation of their protoplasm) were actually killed in the nongerminated stage.

Spores of other fungi occurred occasionally on slides, and it was noted that they would germinate in dilutions in which *Mycosphaerella* conidia would not. For example, in one experiment 4 conidia of *Cladosporium* sp. were found on one slide in the 1–1,000,000 copper sulphate dilution series. These had germinated well with long, branched germ tubes, while the *Mycosphaerella* conidia were only swollen, with mere "buds" of germ tubes. The same thing occasionally was observed with spores of *Alternaria* sp. In figure 2, N, is shown an *Alternaria* spore germinating well in 1–2,000,000 copper acetate, while a *Mycosphaerella* conidium, next to it, did not germinate beyond the "bud" stage. Bacterial growth, also, occurred on the slides in dilutions of copper, which inhibited germination of *Mycosphaerella* conidia. The copper-sensitivity of *Mycosphaerella* conidia, under laboratory conditions, surpasses that of algae. Moore and Kellerman (11, 12) found that Spirogyra, one of the most copper-sensitive algae, made good growth in 1 part of copper sulphate in 200,000 parts of water in the laboratory, while 1 part of copper sulphate in 25,000,000 parts of water was sufficient to kill this alga under natural conditions in water reservoirs.

DISCUSSION

It is evident from the results of this investigation that the explanation for the effectiveness of Bordeaux spray against the strawberry leaf spot lies in the extreme sensitiveness of the conidia of the causative fungus to copper toxicity. It has been shown previously that infection occurs primarily through the under leaf surface. However, because of the low, flat habit of growth of the strawberry plant, and the system of spraying practiced in

Louisiana (small, low-pressure sprayers are used), very little of the spray material reaches the under leaf surface. The action of Bordeaux, therefore, cannot be considered protective in the sense that it protects the sprayed surface from infection. It may more properly be considered as eradicative, since it has been shown that the nongerminated spores are killed when they come in contact with the spray residue. So sensitive are the conidia of *Mycosphaerella fragariae* to the toxic action of copper that germination was completely inhibited when the spores were exposed to fresh or dried Bordeaux for as short a period as 30 minutes and then removed from the sphere of influence of the fungicide and washed. A certain degree of toxicity was apparent even at shorter exposures (Table 7). In tests with soluble copper salts, normal germination was not obtained even in as high a dilution as 0.33 p.p.m. of copper sulphate (0.085 p.p.m. of metallic copper).

It is believed that the results obtained justify the following conclusions relative to the mode of action of Bordeaux spray in controlling Mycosphaerella leaf spot of strawberries: (1) The spray greatly inhibits sporulation on the infected leaves, especially on the upper leaf surface. This reduces considerably the amount of potential inoculum. (2) Conidia that come in contact with a sprayed surface (leaves, or the pine-needle mulch) are killed if sufficient moisture be present to wet the spray residue. (3) Some of the copper that comes in solution from the spray residue may diffuse through the dead tissue of the older spots, or run over the margin of the leaf and reach and kill some of the conidia on the under surface. (4) The limited amount of spray material that may reach the under surfaces of some of the noninfected leaves is probably sufficient, considering the extreme sensitiveness of the spores to copper, to protect them from infection. (5) The spray residue on the upper leaf surface prevents the small amount of infection that has been shown (13) to take place through the limited number of stomata that occur on the upper leaf surface. In the last two instances the action of Bordeaux is protective.

SUMMARY

The purpose of the present investigation was to determine the mode of action of Bordeaux spray on *Mycosphaerella fragariae*.

The spray does not affect the fungus mycelium within the host tissue.

Sporulation on the upper surface of infected leaves is greatly inhibited by the spray. Between 7 and 10 times as many spores were found on nonsprayed as on sprayed leaves. This reduces considerably the amount of potential inoculum.

The conidia were found to be extremely sensitive to copper toxicity. In direct contact with freshly prepared 4–4–50 Bordeaux mixture, germination was completely inhibited by as high a dilution as one part of Bordeaux in 1,000 parts of water. A certain degree of toxicity (as judged by the length of the germ tubes) was evident in dilutions of 1–10,000 and 1–20,000 of 4–4–50 Bordeaux.

No germination was obtained when spores were brought in contact with Bordeaux dried and aged on glass slides (one week to over a year) or with Bordeaux weathered on leaves in the field for 50 days. The nongerminated spores appeared dead (coagulated protoplasm) at the end of 24 hours.

A 24-hour exposure to spores in water on sprayed strawberry leaves, after 10 days' weathering in the field, was lethal.

The spores failed to germinate in the supernatant clear liquid of fresh 4–4–50 Bordeaux. In the supernatant liquid of Bordeaux that had been dried on glass the germination was abnormal (short, curled, gnarled germ tubes).

Lime was not toxic. Excellent germination was obtained in 1 per cent lime smears on slides.

When the conidia were separated from the Bordeaux mixture by a partition of filter paper, it was found that they were either killed or permanently injured by an exposure to 30 minutes or longer, for they failed to germinate when removed from the sphere of influence of the fungicide and washed.

When the spore suspension was connected to dry Bordeaux smears on glass slides by means of narrow water bridges, no germination occurred at distances of 1, 2, or 3 mm. At distances of 5, 10, and 16 mm., germination was initiated, the length of the germ tubes being in direct proportion to the distance, but growth soon stopped. It was evident that germination occurred before sufficient copper had diffused through the water bridge.

Solutions of 1–250,000 copper sulphate, 1–500,000 copper chloride, and 1–400,000 copper acetate were lethal to the nongerminated conidia. In higher dilutions (1–500,000 to 1–1,000,000 of copper sulphate, for example) the conidia germinated with very short "buds" of germ tubes, but no further growth occurred. Normal germination, analogous to that of the checks, was obtained in dilutions of 1–4,000,000 of copper sulphate.

It is concluded that the action of Bordeaux spray in controlling the strawberry leaf spot is mainly eradicative rather than protective in character. The spray (1) inhibits sporulation to a large extent, and (2) kills the nongerminated spores that may come in contact with it. A minor degree of protective action also is recognized.

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THE INFLUENCE OF FOUR MOSAIC DISEASES ON THE PLASTID PIGMENTS AND CHLOROPHYLLASE IN TOBACCO LEAVES[1]

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INTRODUCTION

The mosaic viruses are characterized by their ability to reduce the intensity of the green color in irregular zones in the leaves and sometimes the stems, floral parts and fruits of mosaic-susceptible plants. These irregular zones in contrast to those that remain essentially a normal green produce a mosaic pattern.

Microscopic observations and analyses made by several investigators (1, 2, 3) working with tobacco mosaic have indicated that the chlorophyll content is reduced in the light-green zones. These studies presumably were confined to the common mosaic, which usually induces many light-green areas on the foliage. In the present studies, 4 distinct mosaics, differing in their color or pattern characteristics, were used; one, however, only to a limited extent. In addition to studying the chlorophyll, attention was given to the carotene and xanthophyll and to the enzyme chlorophyllase. A brief abstract of the results has been published (7).

MATERIALS

All of the analyses were carried out with Wisconsin-Havana seed tobacco. The 4 viruses and the mosaics they induce are described in considerable detail

[1] All of the studies dealing with the pigments and chlorophyllase were conducted by the senior writer. The studies relating to the general pathological properties and other properties of the viruses presented in tables 1 and 2 and as set forth in the sections dealing with the detailed descriptions of symptoms on tobacco were conducted by the junior writer or under his immediate supervision. All work was done at the Arlington Experiment Farm, Arlington, Va.

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TABLE 1.—*Types of symptoms induced by four distinct mosaic viruses at the temperatures and on the test plants indicated. All solanaceous species were inoculated after the seedling stage when the plants were well established in clay pots. Culture was in greenhouse or chambers with natural daylight and photoperiods*

Test plants	Common mosaic virus	Yellow mosaic virus (Type A)	Mild dark-green mosaic virus	Mild mosaic virus
Nicotiana tabacum L., Var. Wisconsin-Havana seed	18° to 37° C. *A*[a] 29° to 37° C. *D*	13° to 36° C.[a] *A*[f] 25° to 36° C. *D*	18° to 34° C. *A*	21° to 35° C. *A*
Nicotiana tabacum L., Var. Ambalema	21° to 37° C. *F*[c]	21° to 37° C. *F*[c]	21° to 37° C. *F*	21° to 35° C. *A*
Lycopersicum esculentum Mill., Var. Bonny Best	21° to 27° C. *A*	21° to 29° C. *A* 18° to 24° C. *C*	18° to 34° C. *G* 18° to 24° C. *C, E*	21° to 35° C. *A* 18° to 32° C. *B*
Nicotiana sylvestris Spegaz. and Comes	21° to 32° C. *A*	31° to 34° C. *D, E*	31° to 32° C. *A, D* *C* rarely	
Nicotiana langsdorffii Schrank	15° to 34° C. *C, E* *D* rarely	21° to 34° C. *C, E* *D* rarely	18° to 24° C. *C, E* 31° to 32° C. *D, E*	18° to 32° C. *A*
Nicotiana rustica L.	15° to 24° C. *C* 31° to 34° C. *D, E*	15° to 24° C. *C* 31° to 34° C. *D, E*	15° to 32° C. *C*[d]	21° to 32° C. *A*[e]
Nicotiana glutinosa L.	15° to 24° C. *C* 31° to 34° C. *D, E*	15° to 24° C. *C* 31° to 34° C. *D, E*	15° to 24° C. *C* 31° to 32° C. *C, D, E*	21° to 32° C. *A*[e]
Capsicum frutescens L.	15° to 24° C. *C* 31° to 34° C. *D, E*	15° to 24° C. *C* 31° to 34° C. *D* *E* rarely	15° to 24° C. *C* 31° to 34° C. *D, E*	21° to 34° C. *A*[e]
Solanum tuberosum L. Var. Green Mountain (seedling)	.....	.....	.....	15° to 24° C. *A, D*
Phaseolus vulgaris L. Var. Scotia	18° to 37° C. *C*	18° to 37° C. *C*	15° to 37° C. *G*	15° to 37° C. *G*
Cucumis sativus L. Var. White Spine	21° to 31° C. *G*	21° to 31° C. *G*	21° to 34° C. *G*	21° to 34° C. *G*

[a] The wide ranges of temperature cited do not signify that the temperatures fluctuated between the degrees recorded, but tests were made at several points within these ranges.

[b] At 37° C. mottling appears on 3 to 4 leaves, but subsequent leaves usually show no mottling; near 15° C. mottling is light green.

[c] Virus systemic, but mottling is usually absent in the strain of Ambalema used.

[d] Virus systemic, but erratic, inducing necrotic lesions occasionally on new leaves that are not wiped.

[e] Virus not systemic and no local symptoms at 15° C.

[f] The capital letters in the columns refer to the type of symptoms expressed as indicated below by the corresponding letters. The temperature ranges cited in the columns indicate actual tests and not necessarily absolute limits.

A. Virus systemic, inducing mosaic mottling not lethal.
B. Virus systemic, inducing severe veinbanding, which sometimes produces a mottled effect, not lethal.
C. Virus induces local necrotic lesions on mature and nearly mature leaves when inoculated by wiping.
D. Virus induces local chlorotic spots on mature and nearly mature leaves when inoculated by wiping.
E. Virus systemic, sometimes induces mottling on new foliage, induces necrosis and premature death of part or all of the plant.
F. Very resistant.
G. Immune.

in order that they may be compared and contrasted with some degree of exactness. In table 1 the characteristic symptoms on several plant species are recorded with the temperature ranges over which they were observed. This method was necessary, since temperature frequently modifies symptoms to a degree equaling the modifying influences of certain plant species, and to a degree that confuses certain distinct viruses.

The simple properties of the viruses outside of the plant are listed in table 2.

TABLE 2.—*Notes on the properties of the viruses outside of the living plant*

	Tobacco common-mosaic virus	Tobacco yellow-mosaic virus (Type A)	Mild dark-green mosaic virus	Mild-mosaic virus
Inactivating temperature of fresh non-diluted virus in the plant extract with 10 min. exposure	Near 90° C.	Near 90° C.	Near 86° C.	Near 56° C.
Survival at room temperature (Dry leaf tissue)	Many years	Many years	Many years	3 to 10 days
Survival at $+5 \pm 2°$ C. (Plant extract)	do	do	do	20 to 30 days
Survival at $-8 \pm 1.5°$ C. (Plant extract)	do	do	do	50 to 60 days
Survival at $-17 \pm 1°$ C. (Plant extract)	do	do	do	Over 174 days
Dilution end point in water[a]	10^{-5} to 10^{-6}	10^{-5} to 10^{-6}	5×10^{-4} to 10^{-3}	5×10^{-2} to 10^{-5}

[a] In the case of each virus the fresh plant extract was diluted in distilled water and tested by means of systemic infection in tobacco or *Nicotiana sylvestris*.

The symptoms induced by each virus on Wisconsin-Havana seed tobacco cultured in glass chambers and greenhouses at 22° to 27° C. with natural daylight and photoperiods are given in detail in the following paragraphs in order that similarities and differences may be readily determined from comparable material. This cannot be done satisfactorily on a basis of existing literature.

Common Mosaic

At temperatures near 27° C. the virus induces local chlorotic spots on the young leaves when inoculated by wiping. Vein clearing in the new leaves is the first sign of systemic infection, followed by light-green patches on the subsequent leaves. Vein clearing does not persist. The light-green areas usually involve more than 50 per cent of the leaf area on mature and nearly mature leaves; the darker or normal green areas are broken up into many patches. From 3 to 6 of the apical leaves usually develop an upward roll from 1 to 2 mm. wide along the margins, especially near the tips. In addition to this marginal roll, the leaf as a whole may curve downward producing an

"inverted-spoon" effect. Also, the surface of the leaves may exhibit puckering and the margins may be irregular. The virus mutates (4, 5) in local zones in the plant, the yellow-mosaic mutants inducing occasional small yellow spots on the leaves. This virus has been used in previous studies by the junior writer (4). It was obtained originally from James Johnson and, presumably, is the virus which he later designated *tobacco-mosaic virus 1.*

Yellow Mosaic (Type A)

Local chlorotic spots and vein clearing appear as with common mosaic. The mosaic pattern is creamy white, yellow or yellowish green, and occurs on foliage, stem, and fruits. The mosaic patterns are similar in size and shape to those induced by the common-mosaic virus. Upward rolling of the leaf margins, leaf puckering and marginal irregularity are more evident than in common mosaic. The virus originated as a mutant from the common-mosaic virus used in these studies (4).

Mild Dark-Green Mosaic

Local chlorotic spots and vein clearing appear as with common mosaic. After vein clearing the subsequent young leaves—usually 3 to 5—show upward marginal rolling and stunting, but usually little or no mottling appears. Mottling on subsequent leaves—usually 3 to 5—consists of very small light-green areas, which are increasingly fewer but larger on each successive leaf, until the symptoms resemble the mottling induced on tobacco by Johnson's *cucumber virus 1* supplied by S. P. Doolittle. This stage may be followed by leaves that develop the very small light-green mottled areas.

When mottling is most evident the areas tend to be fewer and larger than is the case with common mosaic. Plants with mild dark-green mosaic have a darker green appearance and frequently they show more leaf puckering and more of the "inverted spoon" effect than plants having the common mosaic or the yellow mosaic.

This virus was collected in *Nicotiana glauca* R. Grah. on the island of Grand Canary. It has never given evidence of producing yellow-mosaic mutants in the junior writer's tests (4).

Mild Mosaic

No local chlorotic spots. Vein clearing as with common mosaic. The subsequent 4 to 6 leaves show diffuse light-green patches, some of which contain darker green islands. Subsequent leaves tend to show small definite light-green mottlings at the tips, at the base or along the margins. These signs tend to continue in 4 to 6 of the apical leaves throughout the life of the plant. As these leaves develop the light green approaches more nearly normal green, producing a diffuse light-green patching that may involve the entire leaf. These patches are not conspicuous and frequently disappear. As leaves approach senility, small dark-green mottlings sometimes appear.

The shape of the leaves remains essentially normal, showing little or no

marginal rolling and no puckering. This mosaic is not so mild on tobacco or on *Nicotiana sylvestris* as is the potato veinbanding mosaic, which was supplied to the junior writer by E. S. Schultz. However, it is less severe on tobacco and on *N. sylvestris* than is the potato "Y" mosaic supplied by T. P. Dykstra. On *N. sylvestris* the 3 viruses induce veinbanding, which persists throughout the life of the plants; but this symptom is little more than a pronounced vein clearing in the case of the true veinbanding type, whereas, in the case of the mild mosaic, chlorosis is more pronounced in the tissue adjacent to the veins and veinlets. The "Y" virus induces severe chlorosis and some leaf deformation on *N. sylvestris*.

Although this mosaic is referred to tentatively as mild mosaic, further studies may show it to be a strain of potato "Y" mosaic or of veinbanding mosaic.

Mild mosaic has given no evidence of mutation in these studies. The virus was collected by the junior writer on plants of growing tobacco at Ephrata, Pennsylvania.

A Condensed Scheme for Differentiating the Four Viruses

The virus of mild-mosaic is inactivated in 10 minutes near 56° C., whereas the inactivation temperatures are considerably higher for the other viruses. This virus does not induce necrotic lesions on any of the plants tested.

The virus of mild dark-green mosaic does not induce necrotic lesions on beans nor does it go to tomato, whereas the viruses of common mosaic and yellow mosaic do.

The virus of yellow mosaic induces local necrotic lesions but no mottling on *Nicotiana sylvestris* at 22° C., whereas the virus of common mosaic induces mottling but no necrotic lesions.

METHODS

Tobacco was selected as the plant with which to work because it is subject to virus diseases of several different types and its large leaves provide ideal material for pigment analysis.

The plants were grown in 8-inch pots in a greenhouse at Arlington Farm. The pots were grouped centrally in the house and partially shaded to provide uniform conditions of light and to prevent sunscald of plants affected with yellow mosaic. Aside from routine care the plants were watered once each week with a $\frac{1}{2}$ per cent solution of calcium nitrate to prevent premature yellowing of basal leaves.

Because of the extreme stunting effect of yellow mosaic, it was necessary to grow at least 10 plants of this type against 6 each of all other types, including healthy plants, to provide sufficient material for pigment analysis. All plants used in a series were selected for uniformity and inoculated when about 6 inches high.

The first pigment determinations were made when the healthy plants were about 18 inches tall. The leaves selected for pigment analysis were picked

at a level about one-third the distance from the tip toward the base of the plant. Leaves at this level were almost completely expanded and showed no symptoms of chlorophyll deficiency due to aging or nutritional causes.

The leaf samples were collected in the morning, usually before 9:00 a. m. The plants were watered heavily the night before to insure uniform turgidity when the samples were taken. Two 10-gram samples—one for pigment analysis, the other for dry weight determinations—were weighed immediately from opposite halves of the same leaves. Midribs and large veins were excluded from the samples by stripping out the interveinal areas by hand.

The method of pigment analysis first used was that of Willstätter as modified by Schertz (8). In the present studies it was found that considerable time could be saved, particularly in the separation of the yellow pigments, by altering the method. Details of the modified procedure have been published (6) and will not be reviewed in detail in this paper.

In accordance with the method cited, all measurements of pigment content were made colorimetrically. Chlorophyll was measured as chlorophyllin; the yellow pigments, carotene and xanthophyll, were measured without chemical alteration. In the absence of fixed color standards, the freshly extracted pigments of healthy tobacco plants were used as standards with which to obtain relative values for each of the mosaic types.

Willstätter's method (9) was used for the determination of chlorophyllase. In making chlorophyllase comparisons involving healthy tobacco and the different mosaic types, errors due to differences in time and environment were avoided by simultaneous extractions of the several samples as in the case of pigment analyses. Each important step in the extraction of a group of samples was completed in a single work day. Details of the method are given below.

The finely ground solids remaining after the complete extraction of the plastid pigments were used as the source of material for the determination of chlorophyllase activity. One-half gram samples of the air-dried solids were placed in 200 cc. Erlenmeyer flasks together with 100 cc. aliquots of freshly extracted chlorophyll. The chlorophyll solution was prepared for each test by extracting 500 grams of fresh, finely ground, healthy tobacco leaves with sufficient amounts of 85 to 90 per cent acetone solution to deliver slightly in excess of 600 cc. of the extract. The filtered extract was divided into aliquots by running it into 100 cc. volumetric flasks from a 600 cc. separatory funnel. The 100 cc. aliquots, after being added to the solids in the Erlenmeyer flasks, were diluted with sufficient water to make a 66 per cent solution (volume basis) of acetone. The amount of water to be added was determined by means of a hydrometer prior to dividing the sample.

After the solution was diluted the flasks were stoppered and set aside in the dark at room temperatures for periods of from 12 to 24 hours. By using some form of constant shaking apparatus the time interval can be shortened. Under the conditions specified, however, shorter intervals than 12 hours in the case of tobacco did not allow for a sufficient conversion of chlorophyll for

accurate colorimetric measurement; longer intervals tended to mask differences in chlorophyllase activities and were, therefore, avoided.

The combined solids and pigment extracts were poured into a glass Büchner funnel and completely extracted with a minimum amount of pure acetone. The solids were then washed with approximately 150 cc. of diethyl ether. In this process a wide-mouth separatory funnel was used in the dual capacity of suction flask and separatory funnel, thus eliminating the need of transferring the solution of the pigments after its separation from the solids.

The combined ether-acetone extracts were shaken vigorously in the separatory funnel to transfer the pigments completely to the ether layer. The ether solution of the pigments was then freed of acetone by careful but thorough washing with distilled water.

After the complete removal of the acetone, the altered chlorophyll was extracted with 0.02N KOH in distilled water to which a few cc. of methyl alcohol had been added. Fifty cc. of the KOH solution were used in the first extraction. The ether extract of the pigments and the aqueous KOH were shaken together vigorously for periods of very short duration. Complete separation of the two solutions was permitted between each shaking. Prolonged vigorous shaking may result in the formation of emulsions that are difficult to "crack."

When the separation of the altered chlorophyll from the unaltered fraction remaining in the ether was complete the dilute KOH extract was filtered through filter paper into a 100 cc. volumetric flask. The ether solution of the pigments was washed several times with a few cc. of KOH solution to remove the last traces of altered chlorophyll. The combined washings were then made up to volume with methyl alcohol. Methyl alcohol was used instead of dilute KOH solution because it has a clarifying effect which facilitates colorimetric analysis. The altered chlorophyll solution is unstable and was, therefore, measured immediately.

The unaltered chlorophyll remaining in the diethyl ether after the removal of the altered fraction may be saponified at this stage and measured as chlorophyllin as a check against the values obtained for altered chlorophyll. Except to determine its feasibility, this procedure was not followed in the present work.

EXPERIMENTAL RESULTS

Plastid Pigments

The plastid pigment data given in table 5 are based on green weight determinations. Green weight was found to coincide fairly closely with area, *i.e.*, when leaf samples were collected on a constant-area basis, their green weights roughly approximated a constant. This same relationship existed for dry weights of healthy plants and all mosaic types not causing severe leaf distortions. The dry weight of yellow mosaic samples, however, was consistently low (Table 3) and pigment comparisons made on a dry weight basis were completely out of line with the visible evidence of chlorosis. Therefore, except in table 4, dry weight comparisons have been omitted.

TABLE 3.—*Weight in grams of 7058 sq. mm. area of tobacco leaf samples from healthy tobacco, common-mosaic, yellow-mosaic, and mild dark-green mosaic-diseased plants*

Sample No.	Healthy		Common mosaic		Yellow mosaic		Mild dark-green mosaic		Month sampled
	Green	Dry	Green	Dry	Green	Dry	Green	Dry	
1	6.54	.61	6.32	.68	7.01	.....	6.57	.....	January
2	6.66	.75	6.69	.74	6.70	.54	6.48	.70	"
3	6.96	.83	6.46	.70	6.65	.62	6.36	.75	"
4	6.63	.81	6.29	.68	6.74	.57	6.50	.71	"
5	7.52	.67	.....	.....	7.12	.61	7.27	.77	"
6	7.55	.82	7.61	.92	7.27	.64	6.93	.88	February
Average	6.98	.75	6.67	.74	6.92	.60	6.69	.76	
Averages in terms of percentage	100	100	95.5	98.6	99.1	80.0	95.8	101.3	

TABLE 4.—*Relative chlorophyll content of leaves of healthy and mosaic tobacco plants, determined colorimetrically, in terms of depth of solution in millimeters*

Sample No.	Healthy plants	Green-weight basis		Dry-weight basis		Month sampled
		Common mosaic	Yellow mosaic	Common mosaic	Yellow mosaic	
1	10.00	10.24	15.32	13.31	15.32	February
2	10.00	13.20	21.68	17.66	22.11	"
3	10.00	13.36	23.41	.....	15.33	March
4	10.00	12.70	23.68	12.70	16.83	"
5	10.00	12.50	20.96	12.68	15.02	"
6	10.00	11.34	15.52	11.08	11.30	"
7	10.00	10.96	18.95	12.08	13.45	April
Average	10.00	12.04	19.92	13.25	15.62	
Averages in terms of percentage	100	83.0	50.2	75.4	64.0	

Typical values for 7 separate pigment extractions, all made during the life of a plant series over a period approximating 2 months, are given in table 4. In this series comparisons are made, both on a green weight and dry weight basis, between healthy plants and those having common mosaic, and yellow mosaic. The values recorded represent averages in millimeters of solution of from 4 to 10 (usually 5) separate readings on the colorimeter and, as recorded, are inversely proportional to concentration.

In table 5 colorimetric values are given that indicate the relative chlorophyll, carotene, and xanthophyll contents of healthy tobacco plants and similar plants affected with common mosaic, yellow mosaic, mild dark-green mosaic and mild mosaic. The plastid-pigment extracts from the healthy plants in each series were used as the standards of comparison. The values have been averaged where more than one analysis was involved. For example, in series 1, table 5, the values given are averages of 4 separate pigment analyses. Averages for the entire series are given at the bottom of the table on both an actual and a percentage basis.

In table 4, values under samples 1, 6, and 7 are considered to be atypical and are, therefore, excluded from the general averages in later tables. In

TABLE 5.—*Relative chlorophyll, carotene, and xanthophyll content of tobacco leaves affected with four types of mosaic (determined colorimetrically in terms of depth of solution in mm., compared with a depth of 10 mm. of solution from healthy plants)*

No. of analyses	Chlorophyll				Carotene				Xanthophyll				Month sampled
	Mosaic				Mosaic				Mosaic				
	Common	Yellow	Mild dark-green	Mild	Common	Yellow	Mild dark-green	Mild	Common	Yellow	Mild dark-green	Mild	
4	12.94	22.42	.....	.....	12.76	23.12	.....	.....	12.72	20.62	.....	.....	Feb.–March
2	14.29	25.44	11.11	.....	15.13	26.93	11.60	.....	14.20	21.09	11.61	.....	May
1	12.46	20.26	10.75	.....	12.74	18.72	10.70	.....	13.14	18.72	11.10	.....	July
1[a]	12.96	24.35	.....	.....	12.36	27.82	.....	.....	13.04	20.14	.....	.....	Sept.–Oct.
1[a]	12.22	18.84	11.04	.....	13.96	24.36	10.56	.....	13.10	22.98	10.38	.....	Dec.
2	15.44	27.35	12.99	13.85	15.45	31.84	13.26	14.34	14.75	24.44	13.52	13.29	March
Average	13.38	23.11	11.47	13.85	13.75	25.47	11.53	14.34	13.49	21.33	11.65	13.29	
Average in %	74.7	43.3	87.2	72.2	72.8	39.3	86.7	69.7	74.1	46.9	85.8	75.2	

1[a] = Two chlorophyll analyses.

explanation, it should be stated that sample 1 was collected early in the life of the plants before the full effects of mosaic were in evidence. Samples 6 and 7 were collected when the plants were approaching maturity and the symptoms of mosaic were becoming masked. In later work fewer pigment analyses in any one plant series were made and the extremes cited were thus avoided.

The chlorophyll, carotene, and xanthophyll content of all mosaic types (Table 5) falls below that of the controls and, though there is a considerable fluctuation in the values from one series of tests to the next, the different mosaics hold their relative positions in all the tests, with the possible exception of mild mosaic. The chlorophyll and carotene determinations place this type between common mosaic and yellow mosaic. On a basis of xanthophyll content, however, mild mosaic lies between mild dark-green mosaic and common mosaic. The actual differences in values are slight and may be of no significance. An insufficient number of analyses were made of mild mosaic leaf tissue to give the values much weight but it is included for purposes of record.

On the average, reduction in the amount of chlorophyll is shown to be accompanied by an approximately proportional drop in 2 yellow pigments, carotene and xanthophyll. The yellowing of tobacco as a result of mosaic thus is clearly due to the partial elimination of chlorophyll as a masking agent rather than to an increase in yellow pigmentation as might be inferred from visual inspection of the plants.

Chlorophyllase Studies

Data pertaining to the measurement of the chlorophyllase activities of healthy and mosaic tobacco leaf tissues are cited in tables 6 to 9, inclusive.

TABLE 6.—*Relative chlorophyllase activity of leaf tissue of healthy and mosaic tobacco plants, as measured colorimetrically, in terms of depth of solution in millimeters*

Series No.	Healthy plants	Common mosaic	Yellow mosaic	Mild dark-green mosaic	Mild mosaic	Number of assays	Month
1	10.00	9.05	3.46	7.40	.....	5	February to March
2	10.00	7.76	3.25	6.40	.....	2	May
3	10.00	11.39	8.35	9.83	.....	1	July
4	10.00	12.18	5.41	11.18	.....	2	December
5	10.00	12.68	6.25	7.42	7.98	1	March
Average	10.00	10.61	5.35	8.45	7.98	.....	
Average in terms of percentage	100.00	94.3	186.9	118.5	125.3		

The values in table 6 show the relative chlorophyllase activity of the ground and extracted leaf tissues of healthy and mosaic tobacco. With the exception of common mosaic, the chlorophyllase content of mosaic plants averages

TABLE 7.—*Relative chlorophyllase and plastid-pigment content of healthy and mosaic tobacco. Values are averages from tables 5 and 6*

Enzyme or pigment	Healthy plants	Common mosaic	Yellow mosaic	Mild dark-green mosaic	Mild mosaic
Chlorophyllase	100	94.3	186.9	118.5	125.3
Chlorophyll	100	74.7	43.3	87.2	72.2
Carotene	100	72.8	39.3	86.7	69.7
Xanthophyll	100	74.1	46.9	85.8	75.2

TABLE 8.—*Relative chlorophyllase activity and plastid-pigment content of the green and yellow areas of yellow-mosaic leaves*

	Green	Yellow
Chlorophyllase	100	123
Chlorophyll	100	67
Carotene	100	86
Xanthophyll	100	98

TABLE 9.—*Relative chlorophyllase activity and pigment content of leaves from near the top, middle, and base of healthy tobacco plants*

	Top	Middle	Bottom
Chlorophyllase	100	52	22
Chlorophyll	100	53	20
Carotene	100	69	20
Xanthophyll	100	61	20

appreciably higher than that of healthy plants. The chlorophyllase activity of yellow mosaic averages about double that of healthy leaf tissue. This is particularly significant when compared with the pigment data in table 5, which show that the chlorophyll content of yellow-mosaic leaves is about one-half that of healthy leaves. The approximate halving of the chlorophyll content by yellow mosaic was, therefore, associated with about a twofold increase in chlorophyllase activity.

Table 7 is compiled from tables 5 and 6 to facilitate comparisons between plastid-pigment and chlorophyllase values. The values for chlorophyllase, chlorophyll, carotene, and xanthophyll in the case of mild mosaic are based on a single assay. All other values are averages of 11 to 13 assays as indicated in the table. With the exception of common mosaic the chlorophyllase activity of the mosaic plants is higher than that of healthy plants. The pigment content of all mosaic types, in contrast, is lower than the controls.

The data in table 8 indicate the relative chlorophyllase activity and plastid-pigment contents of the green and yellow tissues of yellow-mosaic leaves. It will be noted that the yellow tissues contain considerably less chlorophyll, somewhat less carotene, and about the same amount of xanthophyll. In contrast, though of lower chlorophyll content, the yellow leaf tissues are higher in chlorophyllase activity than are the green tissues.

The values given in table 9 indicate the relative chlorophyllase activity and pigment content of leaves from the top, middle and base of healthy tobacco plants. Basal leaves were pale green to yellow. Chlorophyllase activity in this case is almost directly proportional to pigment content. As pigment content falls off, chlorophyllase activity slows down. A halving of the chlorophyll content—middle leaves, compared with the top—is accompanied by an approximate halving in chlorophyllase activity.

The relative chlorophyllase content of different tissues from plants with yellow mosaic were as follows: leaf tissue 100, cortex 14, pith and phloem 9, and roots 9. The amount of change in the chlorophyll extract produced by root and pith tissues was almost negligible. Both root and pith were devoid of chlorophyll, the phloem tissues were very low in chlorophyll, while the leaves contained approximately one-half as much chlorophyll as did the healthy plants.

DISCUSSION AND CONCLUSION

Reduction in chlorophyll content by mosaic was accompanied by an approximately proportional drop in xanthophyll and carotene. The yellowing of tobacco is thus due to the partial elimination of chlorophyll as a masking agent rather than to any actual increase in yellow pigmentation.

These results would seem to differ from those of Elmer (3) who, though reporting a reduction in xanthophyll content, found an approximate doubling of the carotene content in both the dark-green and light-green areas of tobacco mosaic. Presumably, Elmer worked with the common mosaic of tobacco but the discrepancy between his findings and those here reported suggests the possibility that he may have used a virus isolate quite different from the common mosaic used by the writers. This possibility is of interest, in view of the fact that none of the four virus types used in the present studies increased the carotene content. It is possible also that environmental factors influence plastid-pigment production with reference to a given virus.

Owing to the difficulties encountered in word descriptions and to the high cost of colored lithographs it appears that plastid-pigment analyses offer one of the best means for describing the degrees of green and yellow colorations manifested by different mosaics. Some consideration has been given to spectroscopic methods for analyzing the colors on the leaf surface, but several factors of error must be studied before the value of this method is known.

With the possible exception of common mosaic of tobacco, all the mosaics studied tended to increase the chlorophyllase activity of tobacco leaf tissues. The increase in chlorophyllase activity in each case was accompanied by a decrease in the chlorophyll content of the tissues.

In direct contrast to the above relationship when the loss of chlorohyll is due to nutritional causes or to aging of the plant, the chlorophyllase decreases. Whether or not this relationship maintains in plants other than tobacco, or in plants with other chloroses, has not been ascertained. It is

possible that the chlorophyllase level might be used as a quick method for distinguishing certain types of chlorosis.

SUMMARY

A method of determining the chlorophyllase activities of plant tissues based on procedure established by Willstätter is described.

The "green weight" of tobacco leaves was found to be comparable to area as a basis for making plastid-pigment and chlorophyllase comparisons. Dry weight was less comparable to area because of the comparatively low dry weight of yellow mosaic plants. The chlorophyll content of this type, when corrected for low dry weight, was out of line with visible evidence of chlorosis.

The chlorophyll content of tobacco leaves affected with common mosaic, yellow mosaic, mild dark-green mosaic, and the mild mosaic was found to be consistently lower than that of healthy plants.

The drop in chlorophyll content caused by mosaic was found to be associated with an approximately proportional drop in the yellow pigments, carotene, and xanthophyll.

With the exception of common mosaic, the chlorophyllase activity of the ground leaf tissues of mosaic plants averaged appreciably higher than that of healthy tobacco.

A reduction of 50 per cent in the chlorophyll content of yellow mosaic plants, compared with healthy tobacco, is associated with an approximate doubling of the chlorophyllase activity of the ground leaf tissues. The yellow areas were found to be lower in chlorophyll but higher in chlorophyllase activity than the green areas.

In contrast to the above relationship, the chlorophyllase activity of healthy leaf tissues was found to be directly proportional to chlorophyll content. A 50 per cent reduction in the chlorophyll content, middle leaves compared with top leaves, was found to be accompanied by a similar reduction in chlorophyllase activity.

In a comparison of leaf, stem, and root tissues of yellow mosaic plants, the chlorophyllase activity was found to be proportional to chlorophyll content. Chlorophyllase activity was very low in pith and root tissues that were devoid of chlorophyll.

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THE LONGEVITY OF CERCOSPORA BETICOLA IN SOIL[1]

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The source of initial infection of *Cercospora beticola* Sacc., on sugar beets under field conditions is not thoroughly understood. Vestal (6) reported that certain weed hosts may initiate primary leaf-spot infection, while Pool and McKay (4) reported that primary infection was due to the overwintering of the organism on old beet tops. Vestal also suggested that *C. beticola* not only lived but also reproduced saprophytically on dead tissues of the sugar beet and several weed hosts. Massee (1), Pammel (3), and Thümen (5) believed that the spores were able to live for a time in the soil and retain their pathogenicity. The writer, in a few cases, has recovered the organism from old beet refuse.

Continuous cropping to sugar beets markedly increases the prevalence of *Cercospora beticola*. A four-year rotation system reduces the damage caused by this pathogen. Primary and secondary infections usually are sufficiently delayed where a crop-rotation system is followed, so that the accumulative increase of the pathogen during the season, even under favorable environmental conditions, seldom reaches the epiphytotic stage.

Pool and McKay (4) reported that the conidia, when exposed to outdoor conditions, died in 1 to 4 months, but, when kept dry, might live 8 months. The sclerotia-like bodies imbedded in the host tissue lived through the winter when protected in the center of a pile of beet tops or when buried in the soil 1 to 5 inches. Pool and McKay also believed that the organism under the above-named conditions becomes a source of primary infection the following year.

The writer in an abstract (2) reported that *Cercospora beticola* grew well and retained its pathogenicity in 5 different kinds of sterile soil for 27

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months, and that abundant leaf-spot infection developed on cotyledons and leaves of sugar-beet seedlings grown on naturally infested field soil in the greenhouse.

GROWTH OF CERCOSPORA BETICOLA IN STERILE SOIL CULTURES

Cercospora beticola was grown on 5 different soils obtained in the vicinity of Kanawha, Iowa. These soils consisted of peat, basic black loam, acid black loam, black loam low in organic matter, and neutral black loam. The pH varied from 6.0 to 7.4, as shown in table 1. The soil was uniformly

TABLE 1.—*Growth of Cercospora beticola on five different sterilized soils collected at Kanawha, Iowa*

Soils tested	pH	Mycelial growth (av. diam. in cm.)
Peat	6.4	6.4
Basic black loam	7.4	3.8
Acid black loam	6.0	3.6
Black loam low in organic matter	6.9	3.1
Neutral black loam	6.8	5.0

moistened by the addition of water until a friable state was obtained, then placed in Petri dishes and firmly packed, leaving about a 3 mm. space between the level of the soil and the top of the Petri-dish cover. Thirty dishes, 6 replications of the 5 soils, were steam-sterilized and then uniformly inoculated by placing an aqueous conidial suspension of the organism on the surface of the soil in the center of each dish. The sterile, inoculated-soil cultures were placed in a moist chamber to prevent evaporation and incubated at 25° C. The rate of growth of the fungus (Table 1) was calculated by measuring the diameter of the surface growth of the mycelia during ten 24-hour intervals. No quantitative growth measurements were taken, but it was noted that the mycelia uniformly penetrated the soil.

During the early development of the cultures, conidial production was observed in all cases. The best vegetative growth occurred on the peat and neutral black loam. There apparently was no correlation between pH and rate of growth, but the soils that were the highest in organic matter produced the most vegetative growth.

LONGEVITY OF CERCOSPORA BETICOLA ON STERILIZED SOIL CULTURES

Portions of the 5 different soils listed in table 1 were moistened with distilled water to a uniform friable state, and each was used to half fill six 250 cc. Erlenmeyer flasks. After sterilization the soil portions were uniformly inoculated. The cultures were then placed in a large moist chamber for 59 days at a temperature of 24°–26° C. During this time the organism had spread generally through the soil. The cultures were then removed and exposed to laboratory temperature where they soon attained air-dry conditions. From each set of the 5 different types of soil, 1 culture of each was placed outdoors throughout the winter months. During this period the

temperature varied from +15.1° to −27.8° C. A duplicate set, and also a third set consisting of 4 flask cultures of each kind of soil were held under laboratory conditions.

The 2 sets of cultures, 1 of which was kept outdoors, the other in the laboratory, were used to inoculate young sugar beet plants in the greenhouse. At intervals of 18, 27, and 40 months, inoculations of sugar beets were made (Table 2). The method of growing the beet plants, and of preparation of

TABLE 2.—*Period during which Cercospora beticola remained pathogenic in sterilized soil cultures*

Series	Soil culture		Number of plants	
	Number	Age	Inoculated	Diseased
I	1 ck.	18 mos.	45	0
	2[a]	"	57	28
	3	"	60	35
	4[a]	"	56	1
	5	"	54	30
	6[a]	"	36	0
	7	"	55	18
	8[a]	"	47	28
	9	"	77	38
	10[a]	"	46	35
	11	"	50	25
II	1 ck.	27 mos.	70	0
	2[a]	"	50	17
	3	"	47	16
	4[a]	"	58	0
	5	"	60	10
	6[a]	"	40	0
	7	"	62	18
	8[a]	"	48	23
	9	"	70	32
	10[a]	"	65	36
	11	"	39	0
III	1 ck.	40 mos.	40	0
	2[a]	"	43	0
	3	"	36	0
	4[a]	"	32	0
	5	"	39	0
	6[a]	"	38	0
	7	"	48	0
	8[a]	"	45	0
	9	"	39	0
	10[a]	"	31	0
	11	"	42	0

[a] Cultures exposed for 3 months to winter weather conditions.

the inoculum was as follows: beet seed, treated with New Improved Ceresan, was planted in pots of steamed soil. When the plants had reached the 3-leaf stage, they were placed in a moist chamber and sprayed with water, so that dry powdered soil would adhere to the leaves. The plants were then exposed to infection by sprinkling the finely powdered soil culture over the leaves with the aid of a screen. Thirty-six hours later the plants were

removed to the greenhouse bench for the remainder of the incubation period. Leaf-spot readings were taken 12 days after inoculation (Table 2, Series I).

At the end of 27 and 40 months, the same procedure was followed, using the same cultures as in series I. The results (Series II) show that there was a reduction in the amount of pathogenic-fungus material in the 27-month-old cultures as compared with the results obtained in series I, in which the cultures were 18 months old.

At the end of approximately 40 months, the same cultures were again tested for viability. The results presented in series III show that after 40 months apparently no pathogenic-fungus material was present in any of the cultures at the time of inoculation.

LONGEVITY OF CERCOSPORA BETICOLA IN NONSTERILIZED FIELD SOIL

Isolation trials to obtain the pathogen from naturally infested soils by the plate-dilution method were failures, probably because the pathogen is a comparatively slow grower. The method used in the inoculation experiments, namely, sprinkling dry soil on the moistened beet leaves, was successful in isolating the pathogen from naturally infested soil, but a small percentage of the beet plants were killed by sprinkling the nonsterilized soil on the young sugar-beet seedlings.

Later, it was found that by planting the treated sugar-beet seed directly in flats of Cercospora-infested soil, good results could be obtained. The earliest infection occurred on the cotyledons and later on the leaves. The inoculum in the soil was brought in contact with the cotyledons and leaves when the plants were watered, and also when the plants wilted, permitting the leaves to lie on the ground until favorable environmental conditions restored them to normal. The beet leaves apparently can be in a wilted state for many hours without visible injury. Wilting of the leaves frequently occurs under field conditions during unusually hot days. From field and greenhouse observations it has been observed that the beet leaf in this condition collects considerable moisture on the lower surface next to the soil, and this acts as a moist chamber and thereby permits infection, if the organism be present. Initial leaf-spot infection may develop in this manner under what might be assumed to be apparently unfavorable conditions for growth of the organism in the field. A general primary infection thus induced and followed by high humidity or rain usually develops rapidly into a severe leaf-spot epiphytotic over the entire field. The occurrence of leaf-spot epiphytotics, such as described above, are frequently observed under field conditions in Iowa.

In order to study this phase of the problem under controlled conditions, soil was collected in the fall of 1934 from a Kanawha field that had grown sugar beets the previous season. In collecting this soil, the upper 1 inch was removed and discarded. The next layer of 3 to 4 inches was placed in containers and taken to the greenhouse at Ames. Fragments of plant débris were removed. Other soils tested consisted of greenhouse compost, to which

was added an aqueous conidial suspension of *Cercospora beticola*. Still another series consisted of compost without the addition of the organism. Naturally infested steamed field soil was used as the check. The results of this experiment are presented in table 3, series I, II and III. The experiment extended over a period of 20 months. During this time 3 different disease readings were made, the same soil being left in its respective flat, and each of the 3 plantings were made in the same soil.

Treated seed was used to plant 3 rows in each of 3 flats in each series. After each leaf-spot reading, the plants were carefully pulled from the soil so as to leave no host tissue, particularly leaf tissue. The first seedlings in series I emerged January 1, and the leaf-spot reading on cotyledons and leaves was made on January 30 (Table 3, Series I). The 12 flats were then carefully wrapped individually in stiff brown wrapping paper and placed outdoors during the balance of the winter months. On May 1, these flats were returned to the greenhouse, unwrapped, the soil allowed to thaw out, and again planted to sugar beets, in the manner described. On June 17, the data were taken on series II. The results, when compared with those of series I, indicated that there had been some decrease in the amount of *Cercospora* present in the soil. Approximately 6 months later no infection, for some unknown reason, had resulted in the case of the compost soil.

After the second count was taken, the plants were again removed, as previously mentioned in the case of series I. The flats were left on a greenhouse bench for 1 month, then removed to the cold room at 5° C. for 3 months, then placed in a second room at a temperature of −7° C. for 6 months. Immediately thereafter the flats were returned to the greenhouse and left for 2 days, or until the soil had completely thawed and attained the greenhouse temperature. Sugar-beet seed was again sown and, on May 30, the data in series III were completed. The results in table 3, series III, show a marked decline in the population of the Cercospora-infested field soil.

The viable period of this pathogen in sterile soil appears to be rather closely correlated with that period during which the organism can remain in nonsterile soil and still be pathogenic to its host, under the conditions of these experiments.

FIELD INFECTIONS SUGGESTING THE SOIL AS THE SOURCE OF INOCULUM

Dates of the earliest observed primary leaf-spot infection in Iowa over the past several years have been variable. This variability is no doubt influenced by such factors as source of infection, environmental conditions, and crop rotation. In Iowa it has been observed that sugar-beet leaves become infected with *Cercospora beticola*, provided they come in direct contact with the soil. This may be brought about in the seedling stage particularly, just after singling (3- to 4-leaf stage). The disturbance to the plants during the process of singling causes them to lose their turgor and fall to the ground, where they may remain for several days. In this condition, the wilted leaves on the ground collect abundant moisture on the lower surface

TABLE 3.—*Period during which Cercospora beticola remained pathogenic in Cercospora-infested field soil*

Series	Soils and treatments	Age soil from date collection	Plants	Diseased plants	Cotyledon inf.	Leaf inf.	Cot. and leaf inf.	Reisolations
		Months	*Total No.*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
I	*Cercospora*-infested field soil	4	338	71.9	68.9	27.2	23.9	100
	Cercospora-infested field soil, steamed	4	506	0	0	0	0	0
	Compost, plus conidial suspension	4	570	9.2	6.8	1.5	1.5	100
	Compost	4	500	0	0	0	0	0
II	*Cercospora*-infested field soil	9	474	66.7	4.2	37.6	24.9	90
	Cercospora-infested field soil, steamed	9	529	0	0	0	0	0
	Compost, plus conidial suspension	9	416	0	0	0	0	0
	Compost	9	472	0	0	0	0	0
III	*Cercospora*-infested field soil	20	265	5.3	1.6	4.5	1.5	100
	Cercospora-infested field soil	20	301	0	0	0	0	0
	Compost, plus conidial suspension	20	252	0	0	0	0	0
	Compost	20	278	0	0	0	0	0

and in this way set up ideal conditions for infection. Heavy rains may induce a similar condition in the case of young beet plants.

In cases where infection takes place later in the season when the foliage of the plants is mature, it is the outer leaves that become infected first. Frequent observations are necessary in order to observe this condition and to note the relative position of those leaves that bear the initial symptoms. Later, the inoculum is passed to inner leaves and epiphytotic conditions rapidly develop.

On land where beets follow beets and where leaf spot occurs each year, the data indicate that there is built up in the soil an increasing amount of the leaf-spot organism. Primary infection occurs from 2 to 3 weeks earlier on land where beets follow beets and develops into epiphytotic proportions more rapidly under normal weather conditions in Iowa.

In 1932 a 2-acre field of sugar beets developed a severe epiphytotic of Cercospora leaf spot, which defoliated the plants. In 1933 the same field, and also an additional acre immediately bordering it, was sown to beets on April 27; and in this case the entire 3 acres were treated as a unit. Under these circumstances adequate opportunity for dissemination of the inoculum was afforded by the distribution of beet refuse by wind, rain, and cultivation during the usual operations incident to sowing and later to the cultivation of the crop.

In midseason of 1933 a severe epiphytotic of leaf spot occurred on that portion of the field cropped to beets the second season. The severe leaf-spot damage was limited to the original 2-acre area. Approximately 2 crops of leaves were defoliated prior to harvest. The bordering additional acre that had not grown beets the previous season showed only light infection, involving little defoliation.

The yields and sugar percentages taken at harvest indicated the relative severity of leaf spot on the 2 portions of the field. The average sugar percentages and acre yields were as follows: beets following beets: percentage sucrose 9.6 per cent, yield 13.9 tons; first year beets: percentage sucrose 12.2 per cent, yield 23.9 tons. In 1933, there was no way of estimating the fertility differences between these two plots. However, there was no significant difference in yields of corn grown on these same plots during the 1934 season, which was also true when barley was grown on these same plots in 1935. Therefore, it seems reasonable to ascribe the large difference shown above to the differences in severity of leaf-spot infection.

In 1933, the first leaf-spot symptoms appeared in the narrowest spacings of the experimental sugar beet plots. The plants were in about the 6- to 8-leaf stage. Only the lower leaves showed a light amount of spotting. These observations were made just after singling. No additional symptoms appeared until the plants had produced approximately a full set of leaves. At this stage of development, the outer leaves usually come in contact with the soil, and it is largely these leaves that obtain the primary infection from the soil. Later, under favorable conditions, secondary infection spreads to the inner or upper leaves.

Again, in 1933, sugar beets were planted on a 20-acre field that had not been cropped to beets for several years. Detailed data were taken in the field on the presence and amount of leaf spot on 23 one-square-rod plots taken at random over the entire field. On August 25, of the 1682 plants examined, 23.18 per cent were healthy and 76.82 per cent had a light infection, consisting of from 1 to 10 spots per plant. In nearly all cases the spots occurred on the lower or outer leaves. No infection "centers" were present in the fields that might have been responsible for the dissemination of inoculum.

In 1934 the first leaf-spot occurrence was on July 25. The plants were in about the fourth leaf stage, due to late planting. The general but light infection on the lower leaves occurred just after singling, succeeded by heavy rains.

Data obtained in 1935 in which several thousand individual leaf counts were made on approximately 1000 plants in the initial stages of leaf-spot development, showed that the symptoms were present almost entirely on the lower leaves.

SUMMARY

This paper presents evidence showing that *Cercospora beticola* can live in the soil, and that it spreads from the soil to the cotyledons and leaves of the sugar-beet plant.

Cercospora beticola grew well on at least 5 different kinds of soil. At the end of 18 and 27 months, respectively, the organism retained its viability and pathogenicity in sterile soil cultures, while at the end of 9 and 20 months, respectively, using naturally infested field soil, the pathogen retained its virility, but there was a marked decline in the amount of *Cercospora* remaining in the soil at the end of 20 months, as determined by the amount of initial infection.

Abundant conidial production occurred in young soil cultures in all cases.

It was found also that this fungus will tolerate a temperature range of a $+15.5°$ to a $-27.8°$ C. in sterile soil cultures without losing its viability or pathogenicity.

A method is described for isolation of the pathogen from the soil indirectly by means of the host plant.

There apparently is no relationship between pH and the amount of mycelial development at a range of 6.0 to 7.4 in the case of the soils tested in this experiment. However, there was a more rapid growth of the mycelium on the soils containing an abundance of organic matter.

When sugar beets were grown a second year on the same field severely infested with *Cercospora beticola* the preceeding year, leaf spot was very destructive and the yield and percentage of sucrose were markedly depressed. Relatively little infection with a higher yield and percentage of sucrose resulted on adjacent land that had not been cropped to beets for several seasons.

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A SOFT-ROT BACTERIOSIS OF PUMPKIN FRUITS[1]

P. A. ARK AND C. M. TOMPKINS

(Accepted for publication January 20, 1938)

An epidemic of soft rot of pumpkin fruits was observed by the authors in September, 1935, near San Pablo, California. In a preliminary report,[2] the cause was ascribed to a bacterium closely related to *Erwinia carotovora*. Since then, the disease has not been observed in epidemic form, although mild cases occurred in various coastal areas in the State in 1936 and 1937. The purpose of this paper is to discuss more fully the causal agent and its host range.

The disease usually affects only young pumpkin fruits. The affected area, which at first appears water-soaked, soon changes into a soft mass, lacking in mechanical firmness, and possessing a disagreeable odor. Frequently, species of *Fusarium* and *Botrytis*, as well as *Rhizopus nigricans* Ehrenberg, are also found on fruits affected by the bacterial soft rot. A form of *Fusarium javanicum* Koord (kindly identified by W. C. Snyder), often found in association with the soft-rot bacteria, is capable of invading detached fruit in moist chambers without wounding (Fig. 1), but in association with bacteria the damage is greater (Fig. 1). The disease does not affect the leaves, stems, or roots of the pumpkin plant.

Plants Affected.[3] Varieties susceptible to the bacterial soft rot (field observations and greenhouse tests) are as follows: *Cucurbita maxima* Duchesne (Squash—var. Aizu Kuzi, Banana, Boston Marrow, Golden Delicious, Green Delicious, Hubbard Blue, Hubbard Golden, Hubbard Kitchenette, Hubbard Warted, Mammoth Chili, Mammoth Whale, Warren); *C. moschata* Duchesne (Cushaw—var. Aizu Wase (Japan), Australian Bush, Green Striped, Japanese Pie, Kentucky Field, Large Cheese, Shishigatani (Japan)); *C. pepo* L. var. *condensa* Bailey (Pumpkin—var. Boston Creek,

[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California. The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged.

[2] Ark, P. A., and C. M. Tompkins. Bacteriosis of pumpkin fruits in California. Science (n. s.) **84**: 18. 1936.

[3] The writers are indebted to Mr. B. F. Dana, U. S. Dept. of Agriculture, Corvallis, Oregon, for kindly supplying fruits of numerous pumpkin varieties for inoculation purposes.

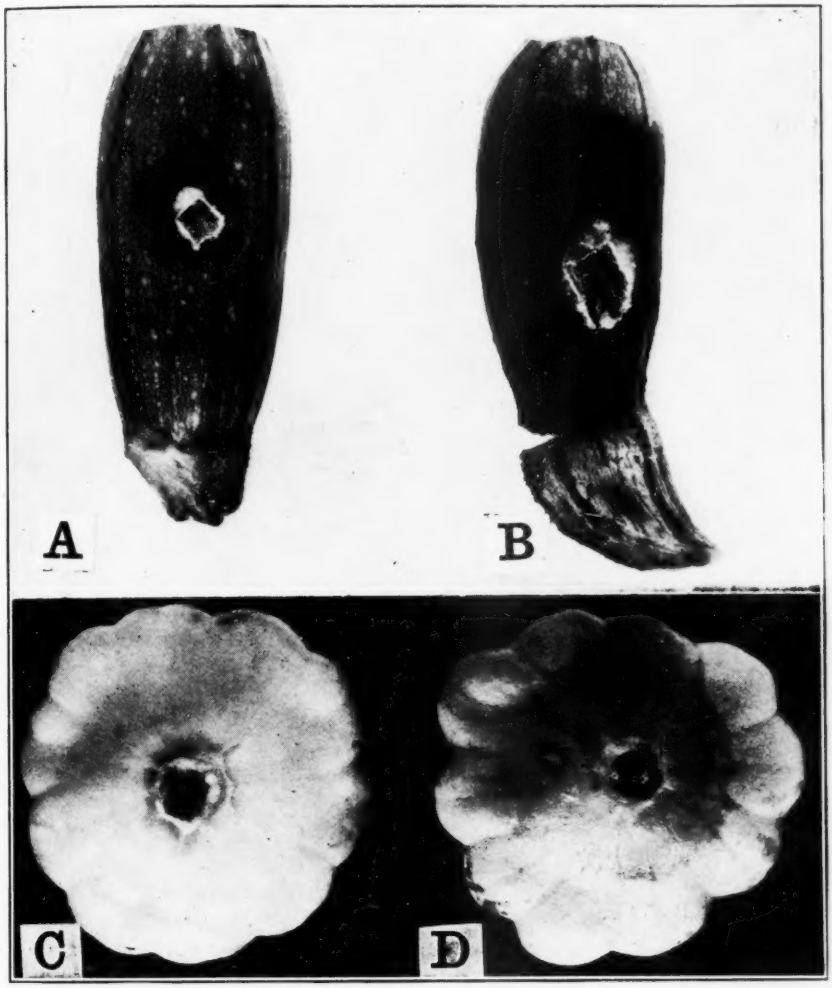

FIG. 1. A and B. *Cucurbita pepo* L. var. *condensa* Bailey (var. Zucchini). C and D. *C. pepo* L. var. *condensa* Bailey (var. Early White Bush Scallop). Blocks of agar with mycelium of the form *Fusarium javanicum* Koord. (A and C). The same fungus as in A and C plus the bacterial organism from pumpkin (B and D). Four days after inoculation. No wound was made in either case.

Cocozelle, Danish, Delicata or Sweet Potato, Early Summer Crookneck Yellow, Early Prolific White Bush, Early White Bush Scallop, Fordhook Bush, Giant Summer Crookneck, Orange Winter Luxury, Pie, Table Queen, Vegetable Marrow Green Vining, Vegetable Marrow Long White Bush, Yellow Crookneck and Zucchini); *C. pepo* var. *ovifera* Bailey (Gourd—var. *maliformis* (Lithuania), *pyriformis verrucosa* (Lithuania), *striata elongata* (Lithuania)); *Lagineria leucantha* Rusby (White-flowered gourd). Early White Bush Scallop (Figs. 2 and 3), Yellow Crookneck, and Zucchini pumpkin fruits were found to be especially susceptible to the bacterial rot under field conditions. Resistance was found only in *C. moschata*, in an unknown vari-

FIG. 2. Natural infection of pumpkin fruits (*Cucurbita pepo* L. var. *condensa* Bailey) var. Early White Bush Scallop.

ety from India, and in gourds (var. *pyriformis striata* and *maliformis latea*) from Lithuania.

The soft-rot organism from pumpkin is also pathogenic to other species of plants when introduced into plant parts by a needle contaminated with the organism. Table 1 shows results of inoculating various plants. Because celery plants (*Apium graveolens* L.) are sometimes severely damaged in the field by the organism (soft rot of crown and roots), numerous varieties of celery were tested in the greenhouse (Fig. 4). Large plants in 6-inch pots were inoculated in the crown and placed outdoors. The following varieties of celery were found to be susceptible: Florida Golden, French Long Top, Golden Phenomenal, Golden Plume, Golden Plume Hybrid, Lagomarsino Special, Long Standing Golden Plume, Tall Paris Golden Yellow, Tethers Special French Tall Strain, and Yellow Hybrid, while Golden Detroit, Utah or Golden Crisp, and Wild Type No. 2 were resistant to the soft-rot bacteria. It is of interest to note that, although several strains of *Erwinia carotovora* from this laboratory failed to cause any effect on *Opuntia* spp., the pumpkin

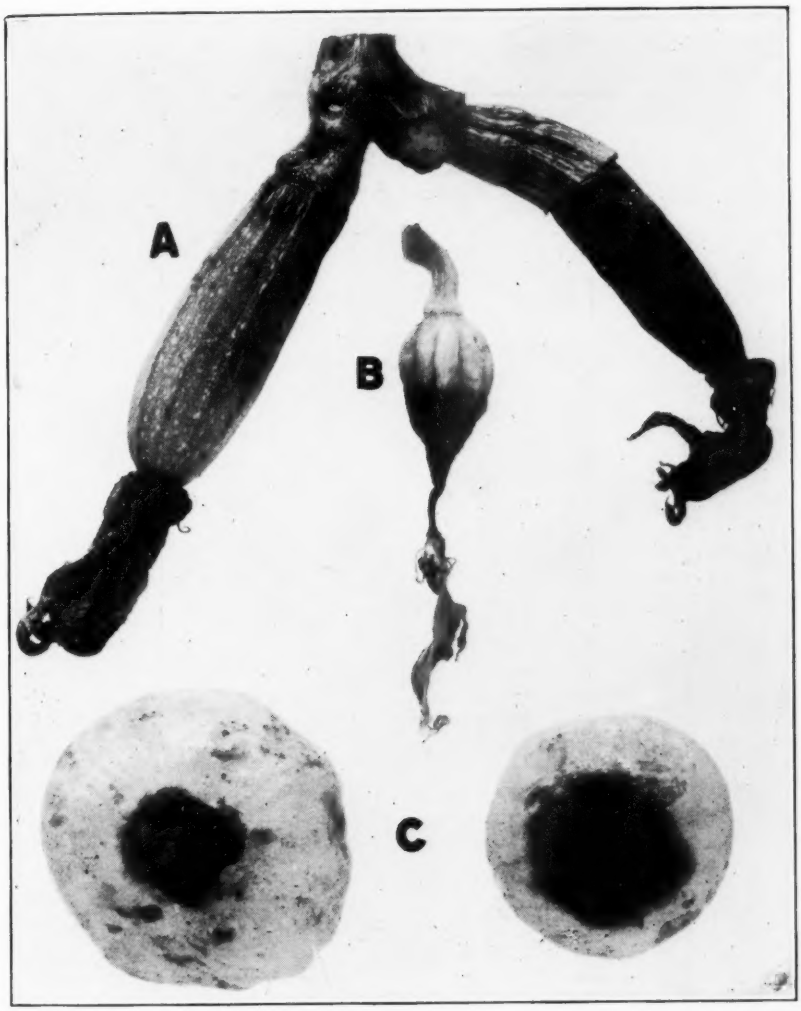

FIG. 3. Pumpkin fruits naturally infected by the pumpkin soft-rot organism. A. Zucchini. B. Danish. C. Early White Bush Scallop.

soft-rot organism disintegrated fleshy parts of some representatives of that genus.

Causal Agent. In the preliminary report by the authors,[4] it was stated that the organism appeared to be related in many respects to *E. carotovora* (L. R. Jones) Holland. Subsequently, more extensive comparative studies were made of cultural and physiological characters of the organism and it has been identified as *E. aroideae* (Townsend) Holland. There were some small differences between certain isolates and the authentic *E. aroideae*. These differences, however, were not great enough, in the opinion of the writers, to preclude the designation of the pumpkin organism as *E. aroideae*. *E. aroideae* from pumpkin appeared to attack hosts that were not attacked by the authentic culture.

[4] See footnote 2.

TALBE 1.—*Inoculations of the soft-rot organism of squash fruit into different plants*

Plant	Part inoculated	Result	Plant	Part inoculated	Result
Allium cepa L.	Bulb	+	*Lycopersicum esculentum* Mill. var. *vulgaris* Bailey	Fruit	+
Ananas sativus Schult	Fruit	+	*Matthiola incana* R. Br. var. *annua* Voss.	Seedlings	+
Apium graveolens L.	Crown[a]	+	*Phascolus limensis* M.	"	+
Beta vulgaris L.	Root	–	*Ph. vulgaris* L.	Pod	–
B. vulgaris L. var. *crassa* Alef.	"	–	*Pelargonium hortorum* Bailey	Cuttings	–
Brassica oleracea L. var. *botrytis* L.	Head	+	*Vicia faba* L.	Seedlings[b]	+
B. oleracea L. var. *capitata* L.	"	+	*Opuntia basilaris* Eng. & B.	Leaf	–
Capsicum annuum L. var. *grossum* Sendt.	Fruit	+	*O. basilaris* Eng. & B. var. *treleasii* Coult.	"	–
Citrus limonia Osbeck	"	–	*O. haematocarpa* Berger	"	–
C. sinensis Osbeck	"	–	*O. inermis* De Candolle	"	–
Citrullus vulgaris Schrad.	"	+	*O. linguiformis* Griffiths	"	–
Cucumis melo L.—var. US45, US619 & US620	"	+	*O. macrocentra* Eng.	"	+
C. melo var. *cantalupensis* Naud.	"	–	*O. mieckleyi* Schumann	"	+
C. melo var. *inodorus* Naud.	"	–	*O. monocantha* Haw.	"	–
C. melo var. *reticulatus* Naud.	"	+	*O. robusta* Wendland	"	+
C. sativus L.	"	+	*O. septacantha* Haw.	"	+
Daucus carota L. var. *sativa* DC.	Root	+	*O. senilis* Parmenteer	"	+
Delphinium ajacis L.	Seedlings	–	*O. vulgaris* Mill.	"	+
Lactuca sativa L.	Head	+	*O. velutina* Weber	"	–

[a] Also petiole.
[b] Also pods.

FIG. 4. A. Celery plant sprayed with the culture of the pumpkin soft-rot organism. Four days after spraying. B. Celery plant inoculated in the crown with an agar-slant growth of the pumpkin soft-rot organism. Two weeks after inoculation.

SUMMARY

The bacterial soft-rot organism of pumpkin in California attacks a large number of pumpkin, celery, and also other varieties of plants under natural conditions.

Several resistant varieties of pumpkin and celery are reported.

The causal organism was identified as *Erwinia aroideae* (Townsend) Holland.

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SPOROPHORE FORMATION BY FOMES APPLANATUS IN CULTURE[1]

HENRY HOPP[2]

(Accepted for publication January 4, 1938)

The formation of typical sporophores by species of *Fomes* growing in culture is not a common occurrence. White[3] obtained fruits of *Fomes applanatus* (Pers.) Wallr. in culture, but they were abnormal in shape and appearance. The failure of mycelium to form normal fruiting bodies in culture was attributed to an insufficient supply of food provided by the small pieces of wood used as a substratum. In the present study, however, it has been found possible to induce the formation of typical fertile sporophores of *F. applanatus* by cultural methods. The strain of *F. applanatus* used in these tests was isolated from the context of a sporophore attached to a fallen log of *Fagus grandifolia* Ehrh. and has been grown since on agar substrata. During this period of over 4 years, the isolate has neither fruited nor shown any significant difference from isolations of this species obtained from several other sources and grown in stock culture under similar conditions.

Cubical wood blocks of *Populus canadensis* var. *eugenie* Schelle., which measured 5 cm. on a side, were used in the present study as a substratum for the fungus. They were partially evacuated of air by soaking them alternately in boiling and cold water until they sank. Each block was then placed on a glass support, 15 mm. high, within a 500-ml. spoutless beaker, and water was added up to the level of the bottom of the block. The top of the beaker was covered with a layer of absorbent cotton, which was held in place by the inverted half of a Petri dish. The culture blocks were then sterilized at 17 lbs.' pressure for 30 minutes. After sterilization, small pieces of mycelial tissue taken from a stock culture were placed on the blocks. The cultures on the wood blocks were then incubated at 28° C. and 100 per cent relative humidity.

After 5 to 6 weeks, when the mycelium was well established in the wood, half the number of blocks were transferred under aseptic conditions to sterilized humidity chambers, the construction of which has been previously described (pp. 19 and 30).[4] The blocks were subjected in these chambers to controlled conditions of aeration and humidity. The chambers were exposed to the diffused daylight of the laboratory. The blocks were so placed inside the chambers that the grain of the wood was oriented in the vertical direction.

Three wood blocks were placed in each of 5 humidity chambers, which

[1] This study was performed while the writer was a graduate assistant in the Department of Forest Botany and Pathology, New York State College of Forestry, Syracuse, New York.

[2] Associate Botanist, Section of Hill Culture Research, Soil Conservation Service, U. S. Department of Agriculture.

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were subjected to different percentages of relative humidity by use of the substances mentioned in table 1. In addition to the 15 blocks placed in these aerated chambers, an equal number of decaying blocks were left in the original culture beakers, and were, therefore, incubated in nonaerated vessels. The experiment was subsequently repeated, so that a total of 60 blocks were used in the course of the entire study, 30 of which were exposed to aeration and controlled humidity; and 30 to nonaeration and 100 per cent relative humidity. The results of the 2 trials were similar.

In the course of 1 to 3 weeks, 14 fertile sporophores developed on 11 of the 30 blocks placed in the aerated humidity chambers. Most of the sporophores were located on the vertical sides of the blocks, but some were on the top surface. In most cases, the sporophores were atypical, in that either a

FIG. 1. Sporophore grown on a wood block of *Populus canadensis* var. *eugenie* that had been subjected to decay by *Fomes applanatus* in culture. The block was exposed to a continuous current of air at 75 per cent relative humidity. ×2.

normal pileus was lacking or the tubes were not oriented in a vertical direction. These atypical forms appeared similar to those described by White. No sporophores appeared during the same period of time on the corresponding 30 blocks incubated in nonaerated vessels.

Four of the 14 sporophores were considered to be normal in appearance and structure. The 4 typical fruiting bodies formed on the vertical sides of those blocks that were subjected to 75 per cent relative humidity (Table 1).

TABLE 1.—*Sporophore production of Fomes applanatus grown on wood blocks in aerated humidity chambers*

Percentage relative humidity	Substance used to control humidity	Total No. of blocks	No. of blocks bearing sporophores	No. of fertile sporophores	No. of typical sporophores
0	Anhydrone	6	0	0	0
35	$MgCl_2 \cdot 6H_2O$	6	0	0	0
53	$CaNO_3 \cdot 4H_2O$	6	3	4	0
75	NaCl	6	5	6	4
100	Distilled H_2O	6	3	4	0

They eventually attained a length of 2 to 3 cm. One of the sporophores is illustrated in figure 1.

As far as known, this is the first time that the formation of typical shelving sporophores of a *Fomes* was induced by cultural methods. In this study, typical sporophores appeared when the cultures were aerated at 75 per cent relative humidity. The environmental conditions conducive to the formation of normal sporophores by *F. applanatus*, therefore, may be defined as follows: (*a*) Exposure of the surface mycelium to ventilation with air of normal oxygen concentration; (*b*) sufficient moisture supply to the mycelium within the substratum; (*c*) continuous but moderate desiccation of the surface mycelium by exposure of the wood block to moist but not saturated air. Although the physiological rôle of these factors was not certain, it appeared that initiation and formation of the sporophores were related to ventilation of the mycelium with "fresh" air (probably oxygen of normal concentration); and that the typical orientation and coloration of the parts of the sporophores were conditioned by the atmospheric humidity.

PHYTOPHTHORA CACTORUM ASSOCIATED WITH SEEDLING DISEASES IN FOREST NURSERIES

BOWEN S. CRANDALL AND CARL HARTLEY

(Accepted for publication January 27, 1938)

During routine isolation work on forest-tree seedlings affected with root rots and damping off of the sore-shin and top-wilt type, *Phytophthora cactorum*, occasionally, has been isolated. Observations on its presence in forest-tree nurseries in this country are of some interest because pathologists in Europe have sometimes reported it, under the synonym *P. fagi* or *P. omnivora*, as causing heavy losses on a great variety of coniferous and deciduous hosts.

In this country Pierce's *Phytophthora* sp. from *Pinus resinosa*, later described by Leonian[1] as *P. pini* n. sp., but closely resembling or identical with *P. cactorum*, has been reported as causing damping off of pine.[2] Inoculation experiments have indicated that it is a weak damping-off pathogen on *Pinus banksiana*,[3] *Pinus ponderosa*,[2] and *Pinus resinosa*.[3] Additional published reports, which indicate that *P. cactorum* is a common cause of dieback and cankers on a number of broadleaf tree species, have been made by various workers as a result of isolations from naturally infected material or on the basis of artificial inoculations.

[1] Leonian, L. H. Physiological studies on the genus *Phytophthora*. Amer. Jour. Bot. 12: 444–498. 1925.

[2] Hartley, C. Damping-off in forest nurseries. U. S. Dept. Agr. Bull. 934. 1921.

[3] Gravatt, Annie Rathbun-. Direct inoculation of coniferous stems with damping-off fungi. Jour. Agr. Res. [U. S.] 30: 327–339. 1925.

———. Germination loss of coniferous seeds due to parasites. Jour. Agr. Res. [U. S.] 42: 71–92. 1931.

See also footnote 2.

According to unpublished reports to the writers, *Phytophthora cactorum* also has been isolated at an Ohio nursery from seedlings of European beech showing symptoms of top wilt[4] and from tulip poplar seedlings with typical sore shin.[5] Some recent findings by the writers are reported in the following paragraphs:

Shortly after emergence *Juglans nigra* seedlings in a North Carolina nursery, both in beds sown broadcast and in rows, suddenly wilted and died. The wilted seedlings were found to have root infections of the soft-rot type. Examination of the blank spaces in beds and rows showed that approximately 50 per cent of the seedlings or seed had been lost before emergence. The infections apparently had started as soon as, or before, the nuts had germinated and had continued as a root rot. In some portions of beds and rows a total loss eventually occurred. Plantings in agar of tissue from the margins of lesions on infected seedlings yielded pure cultures of *Phytophthora cactorum*. Greenhouse inoculations were made on 5-year-old trees by inserting rice, on which one of the isolates was growing, in a cut made at the collar. After inoculation the cut, which extended through the bark to the cambium, was covered with dry sterile cotton and linen tape. Root rot with symptoms similar to those found in the field occurred on 3 of the 5 inoculated trees. No rot resulted from check inoculations made with sterile rice on an equal number of trees.

Seedlings of *Nyssa sylvatica*, *Colutea arborescens* and *Caragana arborescens* at an Elsberry, Missouri, nursery suffered severe losses from infections that appeared to have originated above ground. The injury was characterized by softening of the leaves and upper stem; in the seedlings not too far gone at the time the nursery was visited, the roots were still normal in appearance. *P. cactorum* was isolated from the seedlings of all 3 species after shipment to Washington.[6] Estimated losses were as follows: *Nyssa* 80 per cent, *Colutea* 25 per cent, and *Caragana* 100 per cent. Similar losses occurred at the same time, in the same area, and presumably from the same cause, on species of *Cornus*, *Robinia*, *Acer*, *Prunus*, and *Ostrya*. However, the material of these species reached the laboratory in poor condition and yielded no recognized parasite.

An 80 per cent loss of 4-week-old *Pinus nigra* seedlings sown broadcast in beds was experienced at a single Maryland nursery. The disease was characterized by softening and discoloration of the collar and portions of the root. *Phytophthora cactorum* was isolated from portions of infected seedlings planted in agar.

The strain from *Pinus resinosa* came from a very sandy soil; most of the writers' isolations were from seedlings growing on heavy soils in cool and abnormally wet weather.

[4] Note from Curtis May, Dutch Elm Disease Laboratory, Morristown, New Jersey.

[5] Oral report from Roger Swingle, Dutch Elm Disease Laboratory, Wooster, Ohio.

[6] The identification of the fungus was verified by C. M. Tucker and the occurrence reported in W. E. Maneval's "A list of Missouri Fungi . . ." published in Missouri Univ. Studies, v. 12, no. 3. 1937.

No seedlings of *Nyssa, Colutea, Caragana,* or *Pinus nigra* were available for inoculation. To test the degree of similarity between the strains from them and the so-called *Phytopthora pini,* inoculations were made with isolates of *P. cactorum* from the 4 above-mentioned hosts in comparison with Pierce's original isolate of *P. pini* from *Pinus resinosa* in Minnesota. Ten 3-year-old seedlings were inoculated in a wound at the collar with isolates from each of the species mentioned, and 16 with *P. pini.* *P. pini* killed 15 out of the 16 seedlings inoculated with it; all inoculations with isolates from the other hosts were negative. Check inoculations were also negative.

DIVISION OF FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
UNITED STATES DEPARTMENT OF AGRICULTURE

ADDITIONAL RECORDS OF ROSE ANTHRACNOSE IN THE UNITED STATES

ANNA E. JENKINS AND FRANK P. MCWHORTER

(Accepted for publication February 21, 1938)

Since the identification of rose anthracnose (*Sphaceloma rosarum* (Pass.) Jenkins)[1] in the United States, certain new and more or less historical records of the disease have been obtained and have been employed in a map[2] showing the localities in the United States whence the disease has definitely been reported. The sources of these additional records are given here, except that for Kansas, which is reported elsewhere.[3]

NORTH CAROLINA

When the distribution of rose anthracnose was given in 1932,[4] the only available records for North Carolina were 2 phanerogamic specimens of the commercial rose varieties Alex Tremouillet and Valentine Beaulieu from Biltmore, N. C., collected in June, 1909, by P. L. Ricker.[5] Additional early records of the disease in this State have been obtained through the recent accession of several previously unidentified mycological specimens of rose fungi from North Carolina that proved to be of *Sphaceloma rosarum.* Contributed from the herbarium of the late F. L. Stevens, these are cited as follows: (Locality not given), June 21, 1902, and June 15, 1904, F. L. Stevens; West Raleigh, Sept., 1903 and 1907, F. L. Stevens, and Sept. 21, 1905, S. W. Foster; Bakersville, July 8, 1905; Huntersville, July 27, 1907,

[1] Jenkins, A. E. Rose anthracnose caused by Sphaceloma. Jour. Agr. Res. 45: 321–337. 1932.

[2] Jenkins, A. E., and L. M. Massey. Rose anthracnose. American Rose Annual 1938: 136–141. 1938.

[3] Dearness, J. *Sphaceloma rosarum* (Pass.) Jenkins, as *Glocosporium rosaecola* Dearn. and Barth. nomen nudum. Mycologia 29: 1938. (In press.)

[4] *Loc. cit.*, see footnote 1.

[5] P. L. Ricker Nos. 2640 and 2737. In Economic Collection of the Bureau of Plant Industry. Fragments of these specimens taken by the author in October, 1930, are preserved in the Mycological Collections of the Bureau of Plant Industry (Nos. 68902 and 68903).

and Moorsville, July 29, 1907, F. L. Stevens; Durham, Sept. 10, 1907, Mrs. I. W. Shields.[6] The fungus was again collected at Durham the summer of 1935 by F. A. Wolf (Fig. 1, A).

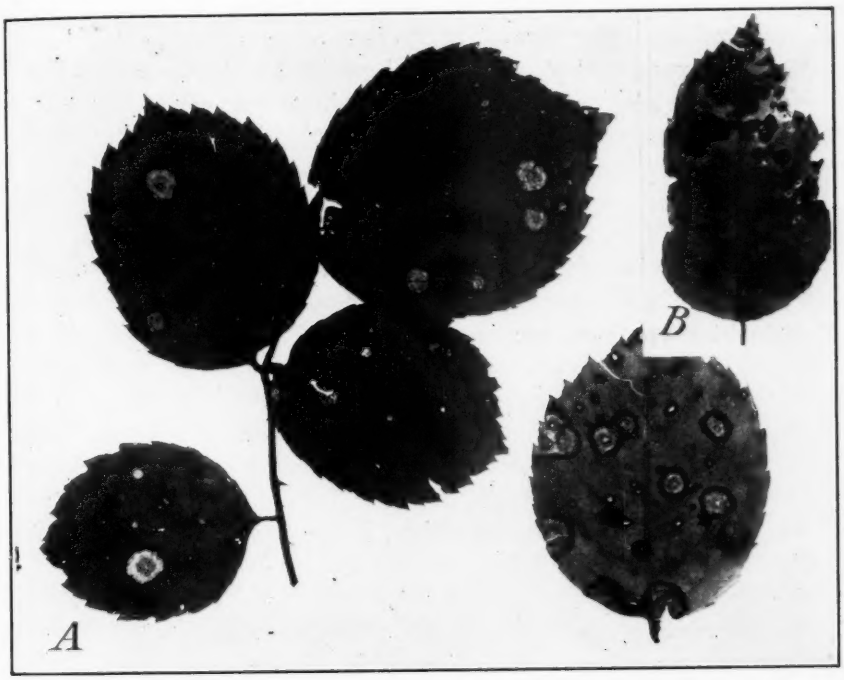

FIG. 1. A. Rose anthracnose, North Carolina. ×1. Photograph by Department of Plant Pathology, Cornell University. B. Anthracnose and black-spot on same leaflet, Oregon, 1937. ×1. Photograph by McWhorter.

OREGON

In the Mycological Herbarium of the Oregon State College is a specimen from a home garden at Albany, 1902, that has been identified recently as rose anthracnose. The only earlier record of the disease in this commercial rose-growing State is from Astoria, 1898,[7] and this is based on the finding of anthracnose lesions on a phanerogamic specimen of a wild rose (*Rosa nutkana* ?) collected by the late F. V. Coville. What is believed to be the same species of wild rose is common in southern Oregon. At Roseburg, the junior writer, in 1934, observed what may be the anthracnose leaf spot on plants growing in an open woodland. During the same year he[8] definitely diagnosed as rose anthracnose a leaf spot of cultivated roses sent to the Oregon State College by a home grower in Oswego. His subsequent observations have shown that, in Oregon, the disease is very common and widespread.

[6] The specimens cited from the herbarium of F. L. Stevens have been divided, and a set has been deposited in the herbarium of the Department of Botany, University of Illinois, and in the Mycological Collections of the Bureau of Plant Industry.

[7] *Loc. cit.*, footnote 1.

[8] McWhorter, F. P. Rose anthracnose in Oregon. U. S. Dept. Agr. Plant Dis. Rep. 139, 1934.

The general occurrence of anthracnose in the rose-growing region of Portland, as well as the need for correct information relative to the disease by commercial rose growers there, was ascertained last July (July 27) when the authors visited 3 different commercial rose plantings in this district and also the municipal Rose Garden of Portland.

The first commercial planting visited consisted of 1-year plants, and the second, of 2-year plants. Each planting was in a separate field, by itself, and was grown as a crop in rotation. In the first planting there was some black spot on *Rosa multiflora*, used as stock, but no anthracnose was found. In the other, there was a small amount of anthracnose on leaves and stems of a certain variety (name unknown) budded as a tree rose.

The show garden of a commercial rose grower in another locality was next visited. There, blooming roses of numerous varieties were abundantly affected with the anthracnose. Black spot was of minor importance (Fig. 1). Representative specimens were taken from the following rose varieties: Betty, H.T.; Betty Uprichard, H.T.; Briarcliff, H.T.; Chaplin's Pink Climber, H.W.; Chatillion Rose, Poly.; C. General McArthur, Cl.H.T.; George Arends, H.T.; Golden Dawn; Golden Emblem; Golden Rapture; Hadley, H.T.; Killarney Brilliant, H.T.; Lord Charlemont, H.T.; Max Krause, H.T.; Mrs. A. R. Barraclough, H.T.; Mrs. Henry Bowles, H.T.; Mrs. Sam McGredy, H.T.; Pink Rapture; and Radiance, H.T.

The Portland Municipal Rose Garden evidently had been sprayed. In this garden rose leaf spots were not abundant, although, during the limited inspection a few anthracnose lesions were found on a rambler rose.

On September 16, at Scapoose, also in the vicinity of Portland, the junior writer made a comprehensive survey of a 2-year-old commercial planting of about 150 acres of which 70 acres were devoted to 2-year-old plants. During the entire growing season these plants were kept dusted with sulphur. Eight to 15 applications are made during the growing season. Leaf spots including those of anthracnose were entirely absent. Considering the prevalence of rose anthracnose in this region, the freedom of this planting from the disease might be taken as an indication of the control of rose anthracnose and other leaf diseases.

MICHIGAN

The occurrence of anthracnose in Michigan, alluded to elsewhere,[9] was first ascertained in 1928, when Eileen W. Erlanson sent diseased specimens from the wild rose collection of the botanical gardens of the University of Michigan to the Bureau of Plant Industry. There appears to have been no subsequent record of the disease in this State until August, 1937, when it was found on leaves of cultivated roses at Mentha by Ray Nelson and the senior writer.

[9] *Loc. cit.*, see footnote 1.

TENNESSEE

From Knoxville, Tenn., in 1934 and 1937 leaves of the Silver Moon and Van Fleet varieties, severely attacked by the anthracnose, were received from J. K. Underwood.

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INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN BROWALLIA

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(Accepted for publication February 15, 1938)

In a preliminary test of the response of *Browallia speciosa* Hook. var. *major* Hort. to infection with tobacco-mosaic virus (tobacco virus 1) two distinct types of disease were found. In one of these, affected tissues became necrotic, but in the other they became chlorotic. The necrotic-type response was local, the other systemic. There were no intermediate types of response.

Tests with plants grown from two lots of seeds, bought in successive years, showed the presence of the two classes with regard to type of disease. In both cases there were more plants of necrotic than of chlorotic type. Only a few plants were tested from the first lot, but the second lot of seeds gave 178 plants of the necrotic type and 84 of the chlorotic type. In both tests plants of the two types were morphologically alike before infection, and could be distinguished only after inoculation.

Necrotic-type plants developed necrotic primary lesions on the second day after inoculation, and then recovered as a result of abscission of inoculated leaves. They showed no chlorotic mottling nor other evidence of systemic infection. Their subsequent growth was normal, and the injurious effect of loss of inoculated leaves was of little importance. They were regularly susceptible to infection upon reinoculation, but were able to free themselves from virus as often as it became established.

Chlorotic-type plants, on the other hand, developed no necrotic primary lesions, but suffered from a systemic disease characterized by chlorosis of the inoculated leaf and all young leaves, and a marked reduction in growth rate. If inoculated when young, they usually died as a result of the severity of the initial phase of the systemic disease. If inoculated when older, they retained more green leaf surface during the period of greatly reduced rate of growth and usually survived to produce an indefinite succession of mottled, distorted leaves and flowers.

The two types of response in *Browallia speciosa* closely resembled those already known in *Capsicum frutescens* L. This suggested that in the necrotic-type plants of *B. speciosa* there might be a single gene capable of

localizing tobacco-mosaic virus in the inoculated leaf, and of freeing the plant from this virus by leaf abscission, as had been previously shown to be the case in *C. frutescens* (1). Hybridization experiments were performed to test this hypothesis.

From the mixed stock of *Browallia speciosa* originally in hand, two pure lines, representing the two observed disease types, were produced through successive self-pollinations. These were then crossed in the following way: A homozygous necrotic-type plant, from which 120 necrotic-type and no chlorotic-type progeny plants had been derived previously by self-pollination, was used as source of pollen for hybridization with a homozygous chlorotic-type plant, from which 29 chlorotic-type and no necrotic-type progeny plants had been derived previously by self-pollination. In the F_1 generation 105 seedlings were obtained, and all were found to be of necrotic type. Among these hybrid plants there was no indication of intermediate-type or chlorotic-type symptoms. This indicated complete dominance of the gene or genes introduced from the pollen parent and responsible for the necrotic type of disease.

Four F_1 plants were self-pollinated. Among the F_2 seedlings derived from them, the following ratios of necrotic-type to chlorotic-type plants were found: 52:17, 52:15, 36:13, 78:27; total 218:72, or 3.03:1. Agreement with a 3:1 ratio, implying one dominant gene controlling necrotic-type response, was satisfactory.

Backcross progeny, obtained by emasculating flowers of 12 plants of the necrotic-type F_1 generation and treating them with pollen from the recessive chlorotic-type parent plant, showed the following ratios of necrotic-type to chlorotic-type individuals: 47:37, 18:12, 14:11, 21:16, 50:46, 41:51, 37:28, 17:18, 53:53, 72:60, 12:13, and 21:17; a total of 403:362, or 1.11:1. This agreed well with expectancy on a monohybrid hypothesis.

The approximate agreement with expectation among individual ratios and totals of progenies obtained by both self-pollination and backcrossing of F_1 plants gave evidence that a single dominant gene was responsible for the necrotic type of response to infection with tobacco-mosaic virus. This dominant gene may be designated as *N* (necrotic-type response to infection with tobacco-mosaic virus), and its recessive allele as *n* (non-necrotic-type, *i.e.*, chlorotic-type, response). This gene *N* in *Browallia speciosa* is entirely comparable, both in its mode of manifestation and in its way of protecting the plant, to the gene *L* previously described in *Capsicum* (1).

Inoculated plants of a backcross set, with segregation in a 1:1 ratio, are represented in figure 1. It will be observed that the recovered necrotic-type plants, in spite of their initial loss of inoculated leaves, had outgrown the systemically infected chlorotic-type plants by the time of photographing. Healthy leaves and flowers from old, recovered, necrotic-type plants and mottled leaves and flowers from corresponding chlorotic-type plants are represented in figure 2.

The simple ratios thus far discussed, obviously pointing to a single dominant gene, controlling necrotic-type response to infection with tobacco-mosaic

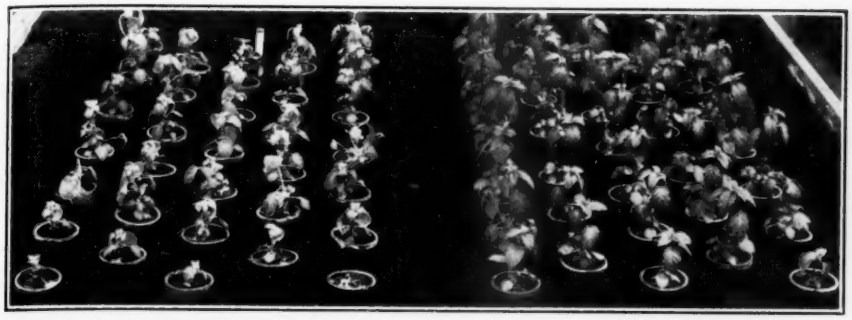

FIG. 1. Backcross progeny, of 32 diseased (*nn*) and 33 healthy (*Nn*) young plants of *Browallia speciosa* Hook., 2 weeks after inoculation with tobacco-mosaic virus. At time of photographing, the recessive-type plants (at the left) were pale and small, as a result of the severe onset of disease; the heterozygotes (at the right) were green and growing rapidly, having lost the virus through abscission of inoculated leaves. Photograph by J. A. Carlile.

virus in *Browallia speciosa*, would adequately represent the situation as known at present, had not two exceptional plants been observed in the course of these experiments. Early in the experiment that had been designed to give a pure line of necrotic-type and a pure line of chlorotic-type plants, one individual was found that gave indication of heterozygosity by segregation of the two disease types among its progeny, and yet did not give the expected 3:1 ratio upon self-pollination. Instead it produced progeny consisting of 104 necrotic-type and 8 chlorotic-type plants (13:1). This plant

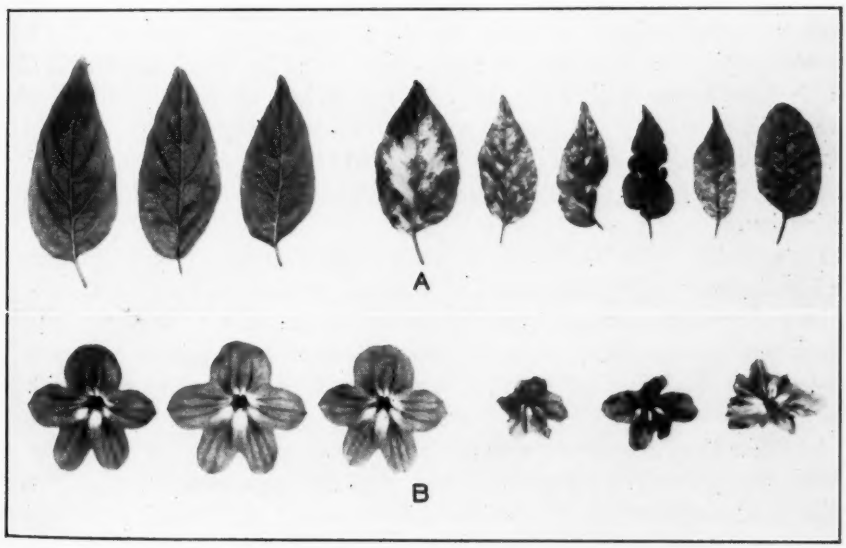

FIG. 2. Leaves and flowers from old plants of *Browallia speciosa* Hook. long after inoculation of the plants with tobacco-mosaic virus. A. Three leaves from necrotic-type (*Nn*) plants after their recovery, and 6 mottled and distorted leaves from chlorotic-type (*nn*) plants systemically affected by tobacco-mosaic disease. B. Three normal flowers and 3 mottled and distorted flowers, from similar plants of each type. Photograph by J. A. Carlile.

was not examined further, but a second plant, one of its own necrotic-type progeny, gave evidence of a similar genetic constitution by producing the same ratio (39 : 3) among seedlings grown from seed of a single capsule produced by self-pollination. A more thorough analysis was made of the genetic constitution of this second aberrant parent plant.

At first it seemed probable that the anomalous 13 : 1 ratios represented dihybrid (15 : 1) ratios. This possibility of a dihybrid type of inheritance was tested by crossing the second aberrant plant with the recessive, chlorotic-type plant that had been used as recessive-type parent in earlier tests. A 3 : 1 backcross ratio would be expected if two independently segregating dominant genes for necrotic-type response were present. In 9 backcross sets having the chlorotic-type plant as seed parent, a total of 843 seedlings were produced and tested; 433 of these were of necrotic type and 410 of chlorotic type, a ratio of 1.06 : 1. Confirmation of the hypothesis requiring two indistinguishable dominant genes was not given by this test, which indicated, on the contrary, that only one dominant gene was involved. In 7 sets representing the reciprocal cross, a ratio of 230 necrotic-type to 228 chlorotic-type plants was obtained. Considered together, these backcross ratios, based on more than 1300 tests, gave substantial evidence that gametes carrying the gene *N* and those carrying its recessive allele *n* were being produced in essentially equal numbers by this heterozygous necrotic-type parent, indicating only a monohybrid type of inheritance. A test of 10 of the 433 heterozygous necrotic-type plants obtained in the first-mentioned of the reciprocal backcrosses was also made by applying pollen from the chlorotic-type plant used previously; the ratios of necrotic-type to chlorotic-type plants obtained in this way were 31 : 22, 60 : 69, 60 : 64, 45 : 34, 64 : 67, 39 : 35, 42 : 50, 14 : 17, 19 : 19, 46 : 40, totals 420 : 417, almost exactly in 1 : 1 ratio. This, again, gave evidence of only one dominant gene for necrotic-type response. Backcross tests of the anomalous plants thus failed to disclose any abnormality of genetic behavior comparable to that observed in progeny obtained by self-pollination.

It seemed possible, therefore, that the discrepancy between the observed 13 : 1 ratio from self-pollination and a simple monohybrid 3 : 1 ratio might indicate the presence of a recessive zygote lethal linked with *n* in the heterozygous plant, but replaced in the recessive chlorotic-type parent by a pair of non-lethal alleles. The 1 : 1 ratios in reciprocal backcrosses had already indicated that no gamete lethal was linked with the gene *n*.

In order to add more data to the original observation, additional sets of plants were grown from seeds obtained from the same aberrant parent as a result of self-pollination. In the interval the parent plant had been cut back, and many new branches had grown out. The following ratios of necrotic-type to chlorotic-type plants were obtained, each representing seedlings grown from seeds of a single capsule on a separate branch of the plant : 24 : 9, 15 : 9, 33 : 15, 25 : 3, 31 : 16, 78 : 30. One of these, 25 : 3, resembled the original aberrant ratio of 39 : 3 in being much greater than a monohybrid 3 : 1

ratio; but all the others were considerably smaller than 3 : 1 ratios. Random distribution about a single mean was not indicated. This occurrence of both high and low ratios, without intermediate values, might be expected, however, if a recessive lethal gene *l* had been linked with the recessive gene *n* in some parts of this plant, but with the dominant gene *N* in other parts. Such a situation might have arisen through somatic crossing-over during the growth of the plant. If *n* and *l* had been linked originally, somatic crossing-over would cause death of all but about the same proportion of *NN* plants as had previously survived among *nn* plants.

In view of the normal ratios obtained from reciprocal backcrosses and these wholly abnormal ratios obtained from self-pollinations, it was concluded that in both aberrant plants a recessive lethal gene was linked with the recessive gene for chlorotic-type response to inoculation with tobacco-mosaic virus, and that the second aberrant plant had also, at least in some of its branches, tissues in which such a recessive lethal gene was linked with the dominant allele, which controls necrotic-type response to infection.

An attempt was made to confirm this conclusion, by further tests of the same sort. Again, some months had elapsed; the aberrant parent plant had meantime been cut back with the result that many new branches had grown out. Eight additional capsules of seeds were obtained by self-pollination of individual flowers on separate branches of this plant during August, 1937. Progenies grown from these seeds were inoculated, and later classified according to their symptoms. The observed ratios of necrotic-type to chlorotic-type plants were 86 : 24, 35 : 8, 42 : 15, 17 : 7, 53 : 24, 96 : 28, 49 : 24, and 24 : 5; total 402 : 135, or 2.98 : 1. It will be observed that there was no confirmation of previous results. No ratio of this group was as high as the aberrant high ratios previously obtained, and only three were as low as the highest among the remainder of the earlier records. Moreover, these ratios were distributed in a random manner about their mean, which agreed almost exactly with a simple monohybrid 3 : 1 ratio.

It is not known whether the lethal factor, for the existence of which earlier tests had given considerable evidence, had actually been lost during the continued growth of the plant, or whether its action had been temporarily nullified. Although the plant under investigation had been cut back at times and allowed to grow out anew, it had not been propagated by cuttings or grafts. All seeds representing progenies that gave evidence of the presence of a recessive lethal gene linked with the gene for type of disease were produced by pollinations at various times during the year other than summer; those giving the simple 3 : 1 ratios were produced by pollinations made in August. It is possible that seasonal factors may have influenced the action of the lethal gene. Since the primary purpose of the present investigation was to study the inheritance of the gene controlling disease type, and since the only available, originally aberrant plant no longer gave an anomalous response, the problem of the linked lethal gene was not pursued further.

In spite of some uncertainty regarding possible successive changes in the genetic constitution of the second aberrant heterozygous plant, all the data

taken together seem to indicate that a single dominant gene determines the necrotic type of disease in *Browallia speciosa*. All backcross data have consisted of approximate 1:1 ratios of necrotic-type to chlorotic-type plants (total 1486:1417, or 1.05:1); self-pollination of heterozygous plants has yielded data consisting either of abnormally high and abnormally low ratios (totals 168:14 and 181:79), or of simple 3:1 ratios (total 620:207, or 3.00:1). Neither the mixed class nor the simple class of self-pollination ratios would indicate more than one dominant gene for necrosis.

Except for the probable existence of a linked lethal recessive factor, therefore, the gene *N* (necrotic-type response to infection with tobacco-mosaic virus) in *Browallia speciosa* is closely comparable to the gene *L* (localization of tobacco-mosaic virus) previously described as derived from the Tabasco pepper and since established in other strains of the species *Capsicum frutescens* (2).

DISCUSSION

Chlorotic-type plants of *Browallia speciosa* were regularly affected, after a single inoculation, with a systemic chlorotic-type disease of such severity as to cause their death, or to render them practically useless for flower and seed production. On the other hand, necrotic-type plants were not seriously injured, even after repeated infection, all invaded leaves being lost by abscission a few days after inoculation.

The necrotic-type plants were consequently disease-resistant in the sense that, under the conditions of the experiments, they effectively restricted virus to inoculated leaves, and so precluded systemic manifestations of disease. It is recognized that excessively high temperatures may induce systemic necrosis in plants capable of localizing tobacco-mosaic virus at ordinary greenhouse temperatures, but such excessively high temperatures are, ordinarily, avoided as deleterious, even for healthy plants. In the present studies temperatures were thermostatically maintained between 70° and 75° F. (21° and 24° C.), so far as possible; the exceptions occurred in summer when temperatures were occasionally as high as 92° F. (33° C.).

In view of the widespread occurrence of the virus of tobacco-mosaic disease, and the ease of its unintentional transfer, it is probable that recessive-type plants may become infected at times when grown for cut flowers. It would be advantageous, therefore, to substitute a homozygous necrotic-type seed stock of *Browallia speciosa*, characterized by resistance to systemic spread of tobacco-mosaic virus, for the mixed stocks or stocks of unknown genetic constitution now in common use.

SUMMARY

Morphologically identical plants of *Browallia speciosa* var. *major* were found to fall into two categories with respect to type of disease when infected with tobacco-mosaic virus. Some plants allowed systemic spread of the virus; others effectively localized it in inoculated leaves. Systemic spread

of virus, in plants of the first class, initially caused chlorosis of young leaves and reduction of growth rate; later it induced mottling and distortion of leaves and flowers. Localization of virus in or near necrotic primary lesions, in plants of the second class, was followed by loss of all infected leaves through abscission. The necrotic-type plants were found to possess a dominant gene *N* in homozygous (*NN*) or heterozygous (*Nn*) condition. This dominant gene was responsible for the necrotic type of disease, and was not found in the chlorotic-type plants (*nn*). The homozygous necrotic-type plants constitute a sub-variety capable of recovering promptly from infection with tobacco-mosaic virus, even when inoculated repeatedly.

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PHYTOPATHOLOGICAL NOTES

Influence of Environment, after Seedling Emergence, on Covered Smut in Barley.—In 1924 Faris[1] reported new and important results with barley covered smut (*Ustilago hordei* (Pers.) Kell. and Sw.), which have not been explained. He inoculated seed of 2 winter barleys by dusting with spores, germinated the seed under controlled soil conditions favorable to infection, and, on emergence, transplanted the seedlings to a field plot or greenhouse in late October. At maturity the following spring, the field plants were smut-free. The greenhouse plants, however, produced considerable smut. To determine the factors involved, the writer, in October, 1936, at Arlington, Va., inoculated formaldehyde-treated seed of Tennessee Winter and Wisconsin Winter barleys by dusting with spores and also by the spore-suspension method.[2] Individual seeds were uniformly sown and germinated in 2-inch pots. On emergence in mid-October, seedlings were transplanted to a greenhouse maintained at 60° F. or above, and to a field plot. In addition, seedlings were transplanted outdoors after first being held in the greenhouse for 2 or 4 weeks after emergence. The winter of 1936–1937 at Arlington, Va., was relatively mild and the field plants suffered no winter injury. The 2 varieties reacted similarly throughout the test. The average results were as follows (each of the following percentages is based upon data from approximately 200 plants): Field and greenhouse plants from noninoculated (control) seed were smut-free. When the spore-suspension method of seed inoculation was used, covered smut in greenhouse plants, and in field plants regardless of the post-emergence treatment, was high and strikingly uniform, varying only from 72.0 to 74.7 per cent (plant basis). However, when seed was inoculated by superficially blackening with spores, covered smut was: (1) relatively low (27.8 per cent) when the seedlings were placed outdoors immediately after emergence; (2) markedly increased (55.1 per cent) as a result of first subjecting the seedlings to greenhouse conditions for 2 weeks after emergence; and (3) further increased (65.3 per cent) after the seedlings were held 4 weeks in a greenhouse before transplanting outdoors. Greenhouse plants from spore-dusted seed produced 63.9 per cent covered smut.

Evidently, the placement and germination of spores beneath the hulls, as occurs in the spore-suspension method of seed inoculation, enabled the fungus, from the time of sowing to seedling emergence, to become sufficiently entrenched within the host tissues to be unaffected by the subsequent external influences. In the spore-dusting method of seed inoculation, however, the spores first must germinate after the seed is sown and the infection hyphae then must advance to the point of attack. It would appear that

[1] Faris, J. A. Physiological specialization of *Ustilago hordei*. Phytopath. **14**: 537–557. 1924.

[2] Tapke, V. F. An effective and easily applied method of inoculating seed barley with covered smut. Phytopath. **25**: 1038–1039. 1935.

under greenhouse conditions these delayed hyphae continued to advance in the tender, succulent tissues. On the other hand, under the rigorous field conditions of late autumn and winter and associated changes in the tissues of the barley plants, further advance of the infection hyphae was blocked and the percentage of smutted plants was greatly reduced. Whether or not this hypothesis may be proved correct, the foregoing results at least appear to indicate that if the fungus has not become well entrenched within the host tissues before seedling emergence, then relatively low temperatures and other outdoor factors immediately after emergence may play a highly important rôle in decreasing the incidence of covered smut in barley. Evidently, this was the factor involved in the above-noted experiment conducted by Faris.—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

A New Pathogenically Distinct Race Derived from a Cross Between Tilletia tritici and T. levis.—The rôle of hybridization in the production of physiologic races of *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn has been the subject of speculation for a number of years. That hybridization may occur between these species and between races within each species has been demonstrated by Flor[1] and later confirmed by Hanna.[2] According to Flor and Hanna the species-hybrid spores were smooth, like those of *T. levis*, but no tests for pathogenicity were reported. In Germany, Becker[3] made hybrids between physiologic races of *T. tritici* and obtained evidence of the inheritance of pathogenicity, as shown by the results of tests with the susceptible variety, Panzer. Thus far, however, no definite evidence has been presented to show that a new physiologic race of either species has been produced by hybridization.

Recently, the writer has obtained results indicating that a new physiologic race of *Tilletia tritici* has arisen from an interspecies hybrid. The physiologic races used as parents in this study were T_0 and L_8, described and numbered by Rodenhiser and Holton.[4] Hard Federation wheat seedlings were inoculated with a combination of 2 sexually compatible monosporidial lines, one of each race, according to the method described by Flor,[1] and grown to maturity in the greenhouse. Infection was obtained and the hybrid mycelium produced F_1 chlamydospores, which were reticulate, though less prominently so than those of the T_0 parent. Seed of Hard Federation was inoculated with the F_1 chlamydospores and planted in the field in the spring of 1936. A high percentage of infection was obtained, and the F_2 provided sufficient inoculum to test for pathogenicity. F_2 chlamydospores, which

[1] Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. Jour. Agr. Res. [U. S.] **44**: 49–58. 1932.

[2] Hanna, W. F. The physiology of the fungi causing bunt of wheat. 5. Pacific Sci. Cong., Canada, 1933, Proc. **4**: 3195–3204. 1934.

[3] Becker, T. Untersuchungen über Sexualität bei *Tilletia tritici* (Bjerk.) Wint. im Rahmen der Immunitätszüchtung. Phytopath. Ztschr. **9**: 187–228. 1936.

[4] Rodenhiser, H. A., and C. S. Holton. Physiologic races of *Tilletia tritici* and *T. levis*. Jour. Agr. Res. [U. S.] **55**: 483–496. 1937.

were morphologically like those of the T_9 parent, were selected and used to inoculate seed of Hybrid 128, Oro, and Hohenheimer and, at the same time, other seed of these varieties was inoculated with chlamydospores of the two parent races. The inoculated seed was planted in the field in the fall of 1936 and smut counts were made the following summer. The bunt percentages were calculated on the basis of total and bunted heads in duplicate 6-foot rows, the number ranging from more than 500 in Hohenheimer to more than 800 in Oro and Hybrid 128 (Table 1).

TABLE 1.—*The percentages of bunt produced by races T_9 and L_8 and a hybrid between these races*

Variety	C.I. Number	Race and percentage of bunt		
		T_9	L_8	Hybrid
Hybrid 128	4512	82.0	80.0	85.0
Oro	8220	3.0	83.0	46.0
Hohenheimer	11458	28.0	0.8	16.0

The bunt percentages presented in table 1 show how distinctly T_9 and L_8 differ in pathogenicity, as well as how the hybrid differs from both parents. The variety Oro is susceptible to L_8 and resistant to T_9, while Hohenheimer is susceptible to T_9 and resistant to L_8. Each of these varieties showed a definite degree of susceptibility to the hybrid, which produced 46 and 16 per cent bunt on Oro and Hohenheimer, respectively. Thus, in view of these results, it seems probable that the hybrid inherited pathogenic properties from both parents. The hybrid was, however, less virulent on Oro and Hohenheimer than were L_8 and T_9, respectively. Nevertheless, the fact that it attacks both varieties to a significant degree distinguishes the hybrid from the parent races. There is also significance in the fact that the hybrid selection resembles *Tilletia tritici* in morphology and was able to produce 46 per cent bunt on Oro, whereas this variety is highly resistant to all of the races of this species described by Rodenhiser and Holton.[5] Furthermore, Hohenheimer is highly resistant to all of the recognized races of *T. levis* and no previously known race of either species has been able to smut both of these varieties.[5] Thus the results presented above seem to indicate that a segregate that possesses the morphology of one parent and certain pathogenic properties of both parents has been selected from a hybrid between *Tilletia tritici* and *T. levis*.—C. S. HOLTON, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in Cooperation with the Washington and Idaho Agricultural Experiment Stations.

Winter Injury of Buxus sempervirens.—In late December, 1928, a severe freeze following a long period of warm weather seriously damaged the large

[5] See footnote 4.

tree boxwoods (*Buxus sempervirens* L.) on one of the estates in the District of Columbia (Fig. 1). None of the other plantings, chiefly *B. sempervirens* var. *suffruticosa* L., were visibly affected by the freeze.

FIG. 1. A. The base of one of the injured tree boxwoods showing the effects of bark cracking and subsequent exfoliation. B. The blighted appearance of the tree boxwood on the left was caused by the effects of the bark injuries on the twigs and trunks. Photographed by G. A. Cromie.

The principal symptom of the injury was the appearance of numerous, irregular, longitudinal cracks or fissures of varying length in the bark on twigs and stems. The more severe injuries generally occurred on the trunks and resulted in a complete separation and exfoliation of the bark. There was no visible evidence of wood splitting. At the time of inspection, made only a few days after the freeze, it was observed that sap flowed freely from incisions made in the bark that was still intact. This indicated that the bark had an abnormally high sap content. In cases where most of the injured bark fell off, the trees soon wilted and died. Observations in subsequent years showed that the large bark cracks usually did not heal and the trees either ultimately died or remained in a weakened and sickly condition.

In order to make a microscopic examination of the injured tissues, discs were cut from a few of the injured stems and stained sections were made from areas that showed a stage of partial separation of the bark. The results showed that the separation was caused by a mechanical rupture that extended tangentially through the phloem and cambium. The microscopic characteristics closely resembled the type shown by plate 21, figure 3, in Grossenbacher's paper on crown-rot of fruit trees.[1] There were no visible mechanical injuries in the woody part of the stem. The individual cells in the bark

[1] Grossenbacher, J. G. Crown-rot of fruit trees: histological studies. Amer. Jour. Bot. 4: 477–512. 1917.

were morphologically like those of the T_9 parent, were selected and used to inoculate seed of Hybrid 128, Oro, and Hohenheimer and, at the same time, other seed of these varieties was inoculated with chlamydospores of the two parent races. The inoculated seed was planted in the field in the fall of 1936 and smut counts were made the following summer. The bunt percentages were calculated on the basis of total and bunted heads in duplicate 6-foot rows, the number ranging from more than 500 in Hohenheimer to more than 800 in Oro and Hybrid 128 (Table 1).

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[1] Grossenbacher, J. G. Crown-rot of fruit trees: histological studies. Amer. Jour. Bot. 4: 477–512. 1917.

tissues showed no evidences of injury from the direct effects of freezing, such as wall ruptures, necrosis, and plasmolysis.

The weather records for 2 months prior to the time of injury showed that the temperature through October, November, and early December was above normal. In the later part of December there was a sudden drop in temperature, which reached 16° F. on the 9th and 20° F. on the 19th and 25th of the month. It is questionable whether these temperatures were sufficiently extreme to produce acute winter injury if the trees had hardened under normal weather conditions.—L. W. R. JACKSON, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

New Records of Anthracnose of Labrador Tea (Elsinoë ledi) and of Snowberry (Sphaceloma symphoricarpi).—In 1933,[1] anthracnose of Labrador tea was recorded in California, for the first time, through the finding of typical lesions on phanerogamic herbarium specimens of *Ledum glandulosum* Nutt. from Mendocino County (Aug., 1882, C. G. Pringle; June, 1903, Jas. McMurphy; May 26, 1913, P. Monnet). The disease is still to be found in this region, as shown by specimens collected there on July 12, 1937, by Lee Bonar (Fig. 1, A), who several years before (July 12, 1933) had found the disease in Humboldt County, adjoining Mendocino County on the north

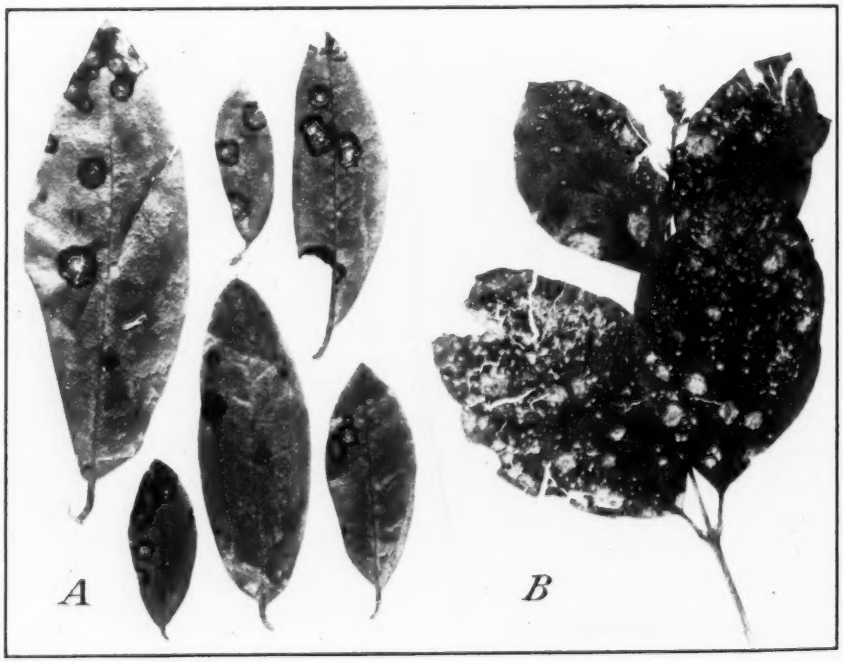

FIG. 1. A. Anthracnose of Labrador tea, California, 1933. B. Anthracnose of snowberry, New York, 1937.

[1] Jenkins, A. E. Additional studies of species of *Elsinoë* and *Sphaceloma*. Mycologia 25: 213–220. 1933.

(Fig. 1). Specimens from both these localities were contributed by Bonar, in July, 1937.

On July 29, 1937, snowberry anthracnose was observed by the writer on *Symphoricarpus* sp. growing spontaneously along a roadside fence in open country northeast of Portland, Oregon. The existence of this disease in Oregon had not previously been known. The only other record from the far west is from California (Sonoma Co.), 1902.[2]

In New York State the disease continues to be present on the old snowberry plantings at Walton and Fishkill previously mentioned,[2] and specimens from these sources were contributed last year (Fig. 1, B). Specimens also were received from Chevy Chase, Md. (October, 1937).—ANNA E. JENKINS, Bureau of Plant Industry, Washington, D. C.

Effects of Bordeaux Mixture on the Control of Yellow Dwarf of Potatoes.—In the work of Black[1, 2] it was shown that the clover leaf hopper, *Aceratagallia sanguinolenta,* is apparently the only vector of yellow dwarf of the potato. An attempt was made in the summer of 1936 to find out whether control of this insect would reduce the percentage of infection. Various concentrations and amounts of Bordeaux mixture were applied to potato foliage at intervals during the season; tubers were selected at random and stored and indexed for yellow dwarf in 1937. The counts of the progeny of sprayed and nonsprayed plants were as follows: of 4813 plants sprayed with Bordeaux mixture, 14 were affected with yellow dwarf, while of 1955 nonsprayed plants, 64 were affected. The results, when treated by the X^2 test, showed a highly significant reduction in the number of yellow dwarf plants for sprayed plants when compared with nonsprayed ones. Such a reduction in the percentage of yellow dwarf (3.3 per cent to 0.3 per cent) may seem small but would be of importance to growers of seed potatoes as sufficient to permit acceptance for certification and reduce materially the labor in roguing. In case of greater spread of the disease, even greater differences might reasonably be anticipated.

Population counts showed no significant differences in the number of leaf hoppers in the sprayed and nonsprayed plots.

The experimental plots were so numerous and so thoroughly randomized that the only known difference between them was the application of Bordeaux mixture. This suggests that copper, taken up by the sprayed plants during or after spraying operations, may play a rôle in the control of yellow dwarf. During the inoculation of the plant by the insect, the presence of the copper in the plant may render the virus partially or totally noninfectious and thus account for the smaller number of plants infected with yellow dwarf.—E. O. MADER and T. C. WATKINS, Cornell University, Ithaca, N. Y.

[2] *Loc. cit.* See footnote 1.

[1] Black, L. M. Some insect and host relationships of the potato yellow dwarf virus. (Abstract) Phytopath. **26**: 87. 1936.

[2] ———. The potato yellow dwarf disease. 1936. Thesis, Cornell University

BOOK REVIEWS

NIETHAMMER, ANNELIESE. *Die mikroskopischen Bodenpilze, ihre Leben, ihre Verbreitung sowie ihre oeconomische und pathogene Bedeutung*. pp. I–V, 1–193. Plates I–VI, text figures 1–47, 1937. W. Junk, The Hague, Netherlands. Den Haag, Nederland.

The author offers a brief introduction covering the universal occurrence and significance of fungi in the soil, the inadequate status of our present knowledge, and a brief presentation of the microscopic and culture methods of study. The microscopic method is regarded as ideal in theory but inadequate in practice. For culture she prefers brewery wort diluted with five times its volume of water to which she adds 5% sucrose and 3% of agar. Other media for special purposes are briefly discussed.

The text appears in 7 parts:

Systematic review of fungous groups, pp. 6–106;
Regional distribution (by nations), pp. 107–148;
Significance in nature, pp. 148–156;
Pathology, pp. 157–165;
Activities, pp. 166–176;
Growth-hormones, pp. 177–179;
Fertility relations, pp. 180–181.

In the systematic section, the species reported in the literature as isolated from soil are taken up group by group, then species by species. No diagnostic descriptions are given. No citation of place of description is given. No reference to the authority for species names or for identification is offered. The species discussed are cited from soil mycological papers without critical consideration of the validity of the use of names. For example, *Penicillium simplex*, which was in reality *Catenularia fuliginea* Saito, is cited from Janke and Holzer. Specific names have their spelling arbitrarily changed; for example, *Penicillium stolonifer* instead of *P. stoloniferum*; *P. terrestrum* for *P. terrestre*. *P. digitatum* is cited as a soil organism, but is probably rarely, if ever, found independently of the Citrus industry. *Aspergillus diversicolor* for *A. versicolor* (p. 41) is a misspelling attributable to Waksman.

Careful scrutiny of the species paragraphs suggests that names introduced by "wir" in the first line of the discussion are applied to organisms known to the author. All others appear in the third person and are probably purely bibliographic. To one familiar with soil mycological literature of the past 20 years, very few items of observation are new, but they have been collected from widely separate publications. Names listed by the various authors appear in the distribution section as reported once or more frequently in Germany or Russia or the United States. No critical examination of the use of names is reported or evident, since such examination would have excluded some usages appearing.

Section 2

In the section on regional distribution the material already used, species by species, is rearranged to produce lists of species found in particular countries. Species are grouped in relation to climate, to forest, or to meadow, without critical analysis of the reason of sources of the original lists.

The discussion of the place of particular species in nature drops the restriction to soil indicated as the purpose of the book and discusses the presence of the same fungi upon other natural substrata. Again, the species selected are those already cited as found in the soil, and other substrata are indicated as also discussed in literature.

The soil as a reservoir of plant pathogens brings forward many of the same specific names cited from the same sources.

The activities discussed for soil fungi include the breakdown of celluloses, hemicelluloses, pentosans, pectins, lignins, tannins, lipases, fermentation of sugars, and proteolytic activity.

The author explicitly disclaims completeness in presenting what is largely a compilation of existing literature. This does not justify inaccuracies in citing proper names such as Traan for Traaen; E. Lennan for Ethel McLennan; Raman for Ramann; Klebhahn for Klebahn; and there are more. These are not misprints, for they appear in the bibliography, as well as in the text.

The author has compiled a large amount of material that has been arranged arbitrarily for her purposes. For one entirely unfamiliar with soil mycology, the lists given may have some value. As a basis for a subsequent series of papers by the same author or by others, it may be convenient as a point of departure. One is compelled to doubt whether the bibliographic rearrangement of groups in *Penicillium* and *Fusarium*, many of which she has obviously never seen, has much use to those attempting to study material in culture.

Unless a proposal for reclassification is fully supported by morphological and cultural data, the user is entitled to the exact citation of at least one standard description of each species discussed.—CHARLES THOM, Principal Mycologist, Bureau of Plant Industry.

DENNIS, R. W. G. and D. G. O'BRIEN. *Boron in Agriculture.* 98 pp. Research Bulletin No. 5, West Scotland Agricultural College. Sept., 1937.

A review of the literature dealing with effects of boron on plant growth, especially crop plants in the field, is the main theme of this publication. Approximately 190 citations are given. The occurrence of this element in different plant materials, soils, rocks and minerals is given insofar as the information is available. The boron content of rocks varies, being least in igneous rocks and greatest in marine argillaceous sediments. Soils also vary but boron deficiency in soils may be primary or induced. Lime and water content of soil are important factors in the availability of this element.

Striking growth effects are described for more than 20 agricultural crop plants under field conditions. The heart and dry rot of the sugar beet with which *Phoma betae* was originally associated has definitely been shown to be due to boron deficiency. A disorder of turnips and swedes characterized by an internal browning or mottling of the flesh has been referred to by a variety of names in different countries but this author prefers to designate it as brown heart. Different organisms have been isolated from affected swedes but they now appear not to be the primary cause of the disorder. Some strains of the swede appear to manifest some resistance to brown heart but this is associated with the ability to form more extensive root systems. Tobacco is another plant belonging to a different family that manifests striking symptoms due to boron deficiency as does the tomato, another member of the same family as tobacco.

Plants belonging to the grass family appear to have a lower boron requirement than most of the other crop plants studied. However, it has been found to be essential for most grasses studied. It produces effects, according to one worker, on sugar cane that resemble Pokkah boeng disease associated with *Fusarium moniliforme* Sheldon. Excess boron has been reported to favor spot blotch (due to *Helminthosporium sativum*) on barley in sand cultures. Boron manuring has been reported to increase the resistance of wheat to rust (species not stated) and when used in small amounts in control cultures it has increased the resistance of *Agrostis tenuis* to attack of *Fusarium nivale*. The control of "Cracked stem" in celery by the use of 10 pounds of commercial borax per acre has been reported from Florida. A dressing of 30 pounds per acre was found to be toxic.

The effect of boron deficiency on the legumes is striking. Cotton grown under conditions where boron was deficient exhibited striking growth manifestations, possibly the most significant of which was shedding of flower buds and young bolls. Citrus, lettuce, and flax also manifested striking growth effects due to shortages of boron. The effect of boron deficiency on the development of the apple fruit producing what is known as drought spot and corky core have been reported from different parts of the World.

A summary table is presented for recorded symptoms due to boron deficiency with crops studied and another for quantity of boric acid, 6½ to 13 pounds per acre, or borax, 10 to 20 pounds per acre, necessary to apply in different parts of the World to prevent deficiency on the various crops previously mentioned. It is pointed out that it is difficult to obtain uniform distribution in the field of the small quantities which are required. It is to be recognized that excessive quantities of boron are toxic and, therefore, are to be avoided.—J. E. MCMURTREY, JR., Bureau of Plant Industry, Washington, D. C.

OSKAR ECKSTEIN, ALBERT BRUNO and J. W. TURRENTINE with the collaboration of G. A. COWIE and G. N. HOFFER. *Potash Deficiency Symptoms.* 235 pp. 41 figs. 54 col. plates. Verlagsgesellschaft für Ackerbau M.B.H. Berlin, 1937.[1]

The student of phytopathology is now fairly well supplied with texts and monographs dealing with parasitic diseases of plants and to a lesser degree with their viroses, but there is still much to be desired in the way of usable and up-to-date discussions of the nonparasitic diseases of plants, although the latest editions of Vol. 1 of Sorauer and of Heald's Manual of Plant Diseases have improved the situation immeasurably. This lack is espe-

[1] Can be secured from B. Westerman & Co. Inc., 24 West 48th Street, New York, at $2.25 per copy.

cially notable with respect to the symptomology and pathological anatomy of plants affected by that important group of deficiency diseases resulting from the lack of or unavailability of the more common soil elements, a subject to which Sorauer devotes about 50 pages and Heald about 13 pages. The appearance, therefore, of a monograph well illustrated with black and white prints and colored plates dealing with the symptoms of potash deficiency should be welcomed by all students who desire to become more familiar with this much discussed but little understood field. The volume under discussion consists of 2 parts with concurrent German, French, and English texts, the first of which is devoted to a general discussion of potash deficiency symptoms, a detailed description of the external symptoms, and the modifications of the inner structure of typical plant organs resulting from potash hunger, and a discussion of the secondary effects of potash deficiency with reference to plant diseases, pests, and climatic factors. The second part of the book deals with symptoms of potash deficiency in specific crops and is well illustrated with photographs and with 54 colored plates prepared from color photographs and colored drawings illustrating a wide variety of normal and potash deficient crops. Included among the illustrations are a few photomicrographs illustrating the effects of potash deficiency on the anatomy of the plant. In addition to the text and numerous illustrations, a subject index and a bibliography of 209 titles also are included.

For the student or worker who would become familiar with the subject of "Potash Hunger in Plants" or who is interested in the interrelationships of potash hunger and other diseases and pests of agricultural plants, this little volume should prove an invaluable and authoritative introduction, especially since the 3 authors of the book and their 2 collaborators have devoted themselves for many years to the problems of potash nutrition. That the preparation and publication of this monograph has been inspired by the scientific bureaus of the various potash-producing syndicates is quite evident. Fortunately, the book seems free of the commercial bias that often seriously detracts from such publications.—V. H. YOUNG, Department of Plant Pathology, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

ANNOUNCEMENT

The Pacific Division of The American Phytopathological Society will hold its annual meeting in Balboa Park, San Diego, California, June 21–23, 1938. In addition to the presentation of papers, occupying four half-days, a symposium on the teaching of plant pathology will be held. An afternoon will be devoted to excursions to near-by points of interest and, following the meeting, tours to Imperial Valley or to Ensenada, Mexico, will be arranged if desired. Pathologists from all sections are invited to participate in the meetings and to present papers.—L. D. LEACH, Secretary-Treasurer, Pacific Div. of American Phytopathological Society, Davis, California.